SÃO PAULO STATE UNIVERSITY – UNESP

JABOTICABAL CAMPUS

THE ROLE OF PHYTOCHROME B1 OF TOMATO (*Solanum lycopersicum* L.) IN THE REPRODUCTIVE STAGE DURING DROUGHT STRESS

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TÍTULO DA DISSERTAÇÃO: THE ROLE OF PHYTOCHROME B1 OF TOMATO (*Solanum lycopersicum* L.) IN THE REPRODUCTIVE STAGE DURING DROUGHT STRESS

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AUTHOR'S CURRICULUM INFORMATION

Carlos Alberto Silva Junior – was born to Carlos Alberto Silva and Odilza Ana da Costa Silva, on January 4, in 1995, in Cuiabá, Mato Grosso, Brazil. He joined the Agronomy course at "Federal University of Mato Grosso – Cuiabá campus" in November 2012. During his graduate course, he was monitor in the discipline of forage production from the Department of Animal Science and Rural Extension in 2016. He was member of study groups that aimed to discuss the most advanced knowledge published in papers, such as NERP (Ruminants and Pasture Studies Nucleus) and NESA (Agronomic Solutions Studies Nucleus). He was a Scholarship holder in the scientific initiation modality by the National Council for Scientific and Technological Development (CNPq) during three consecutive years, from 2014 to 2017. In the last semester, he did an agricultural internship in the United States of America, where lived and worked in a livestock farm named Reynolds Livestock between May 2017 to May 2018 and participated in lectures at Iowa State University. From November 2018 to February 2019, he carried out a curricular internship in a soybean farm located in Tabaporã – Mato Grosso, named Lunil Farms I. He obtained the title of Agronomist Engineer in May 2019. In August 2019, he started his master's course in the Graduate Program in Agronomy (Crop Production), at the "São Paulo State University – Jaboticabal campus". The master's project was funded by CNPq and São Paulo Research Foundation (FAPESP), under the advising of Prof. PhD. Rogério Falleiros Carvalho and co-advising of Prof. PhD. Luiz Fabiano Palaretti.

“Explaining nature is too difficult a task for any man or for any time. It's much better to do a little and definitely leave the rest to the others who come after you”

Isaac Newton

DEDICATION

I dedicate this work to my parents, Carlos Alberto Silva and Odilza Ana da Costa Silva, my endless sources of inspiration, for all the support, care, and advice during my journey.

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SUMMARY

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THE ROLE OF PHYTOCHROME B1 OF TOMATO (*Solanum lycopersicum* L.) IN THE REPRODUCTIVE STAGE DURING DROUGHT STRESS

ABSTRACT – Photoreceptors are primarily known as key photomorphogenic modulators of various physiological events during plant development. Although there are different groups of photoreceptors, the phytochrome B (phyB) family mediates developmental responses in a wide range of plant species, from seed germination to flowering. In addition, these molecules also regulate abiotic stress acclimation responses, such as salinity, drought, low/high temperature, high light, and heavy metals. The signalling pathways mediated by phyB could enhance plant resistance to environmental stresses, as *phyB* mutants reduced leaf transpiration through lowering of stomatal conductance, increased the antioxidant system, enhanced protective pigments, and increased the expression of genes related to plant stress acclimation. Therefore, the scope of chapter one is to compile and discuss the evidence on abiotic stress response in plants that are modulated by the phytochrome type B family. In addition, chapter two aimed to elucidate the responses mediated by phyB1 in tomato fruit production comparing the effects of drought stress in vegetative and reproductive stages. The water deficit treatment was performed in two different stages growth: in vegetative (start at 26 days after sowing [DAS]); end at 36 DAS) and reproductive (start at 33 DAS; end at 41 DAS) stage growth.

Keywords: fruit yield, fruit quality, evapotranspiration, red-light influence, source-sink relationship, water deficit

CHAPTER 1 – Phytochrome type B family: the abiotic stress response signaller in plants

1. INTRODUCTION

Light controls plant development via complex photoreceptor systems that perceive different wavelengths of light. The light causes changes in photoreceptor molecule conformation, allowing it to signal to the nucleus and regulate transcriptional activity of the genome (Voitsekhovskaja, 2019). The activation of photosensory receptors by light triggers responses in several processes during the plant life cycle from seed germination to flowering (Casal, 2013). For example, cryptochromes and phototropins are photoreceptors that primarily detect wavelengths of blue light (300-500 nm) (Christie et al, 2015; D'Amico-Damião and Carvalho, 2018) and were reported as a typical regulator of flowering (Guo et al., 1998) and stomata opening (Inoue et al., 2010), respectively. The photoreceptor UVR8 (UV resistance locus 8) perceives and signals ultraviolet (UV)-B light, modulating plant growth under UV-light. UVR8 interacts with the E3 ubiquitin ligase complex that is also regulated by other photoreceptors (e.g., constitutive photomorphogenic 1 [COP1]), mediating responses, such as hypocotyl length reduction, under UV-B light (Favory et al., 2009). However, the phytochrome (phys) family contains well-characterised photoreceptors, showing absorption peaks in red (R; ~660 nm) and far-red (FR; ~ 730 nm) light wavelengths. Phytochromes are dimeric proteins (~124 kDa) (Jones and Quail, 1986) covalently linked to a phytochromobilin, which is a linear tetrapyrrole with an open chain that acts as a chromophore (Rockwell et al., 2006). Studies concerning phys began in the 1950s, showing that R irradiation induced germination of lettuce (*Lactuca sativa*) seeds, while FR reversed this induction (Borthwick et al., 1952). The interconvertible property of phys triggers plant responses with the active (Pfr – Phytochrome far-red) and inactive (Pr – Phytochrome red) form present after exposure to R and FR, respectively (Rockwell et al., 2006). Some decades latter phy gene sequences were described in Arabidopsis (*Arabidopsis thaliana*) plants and five phys genes were identified, including *PHYA, PHYB, PHYC, PHYD* and *PHYE* that encode their own apoproteins (PHYA to PHYE) (Clack et al., 1989). Biosynthesis of phyA to phyE occurs when each apoprotein binds to its respective chromophore. Phylogenetically, phy

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arose from gene duplication of an ancestral phytochrome gene, but among plant species, different phy types can be found and have not undergone the same phylogenetic evolution. Since their split 140-200 million years ago (Mya), monocots and dicots began evolving independently, and presented separation of *PHYA* from *PHYC* and *PHYB*/*D* from *PHYB/E* (Alba et al., 1997; Mathews and Sharrock, 1997). For example, phyA to phyC is present in rice plant (*Oryza sativa*) (Dehesh et al., 1991) and phyA, phyB1, phyB2, phyE and phyF in tomato (*Solanum lycopersicum*) (Alba et al., 2000). Briefly, some phytochrome subfamilies control similar traits, such as the primary function in seedling de-etiolation of Arabidopsis and tomato mediated by both phyA and phyB (Casal, 2013). However, the individual role of phys, even in the same species, seems to be truly intricate because phyB1 and the homologous phyB2 originated by gene duplication and have non redundant roles. For example, in maize (*Zea mays*), phyB1 plays a substantial role in seedling traits (e.g, hypocotyl length) and phyB2 is fundamental to repress flowering under long-day (LD) photoperiods (Sheehan et al., 2007). In tomato, phyB1 showed a major role in light and auxin responses (gravitropism and phototropism), while phyB2 acted antagonistically to phyB1, promoting photosynthesis (Carlson et al., 2020). Thus, regarding these multifaceted aspects of the phyB family, it is not a surprise that the responses modulated by these photoreceptors include complex signalling pathways.

The activation of phy causes changes in molecule conformation that triggers the phy-regulated photomorphogenic responses. Upon light excitation, the phy molecule suffers an isomerization, which induces a conformation change that exposes nuclear localization sequences allowing the active phys (Pfr) to migrate to the nucleus (Hiltbrunner et al., 2005). When accumulated in the nucleus, Pfr inhibits the transcription of protein groups that act as repressor of photomorphogenesis (e.g., COP/DET/FUS, constitutive photomorphogenic/deetiolated/FUSCA; suppressor of PHYA1 [SPA1]; and phytochrome interacting factors [PIFs]) (Favero, 2020; Lau and Deng, 2012; Leivar and Quail, 2011; Xu et al., 2015), allowing the accumulation of many transcriptional factor (TIFs). Thus, these TIFs promote photomorphogenesis (e.g., HY5 – elongated hypocotyl 5) and trigger the expression of various genes, which leads to the modulation of phytochrome-mediated traits as cited above (Huang et al.,

2014; Balcerowicz, 2020). Recently, it has been observed that responses to abiotic stress are related to photomorphogenesis repressors, such as COP1 and PIF (Kim et al., 2016; Qiu et al., 2020). Thus, it is not surprising that phys were previously described to regulate responses to diverse abiotic and biotic stresses (Carvalho et al., 2011a) such as salt stress (Indor et al., 2007), drought stress (Kraepiel et al., 1994), low and high-temperature stress (Williams et al., 1972; Foreman et al., 2011) and high light (Wellmann et al., 1984). Highlighting the phy type B family show some similar responses among species and many research groups have targeted their efforts to understand phyB-modulated stress responses, because such responses could be altered in the same species, e.g., phyB1 and phyB2 gene duplication controlling different stress acclimation responses (Arico et al., 2019; Gavassi et al., 2017; Kreslavski et al., 2015; kwon et al., 2018; Yoo et al., 2017). Therefore, in this review we summarized the phyB-modulated stress responses to elucidate future research for plant breeding.

2. LITERATURE REVIEW

2.1. Drought stress

Drought stress is a major limiter of crop production and has become even more worrying because of rapid global climate changes in recent decades (Lesk et a., 2016; Garcia e al., 2020). Plants naturally display resistance mechanisms that help them to cope with drought conditions, such as alteration in morphological, physiological, and molecular characteristics (Hussain et al., 2018). The mechanisms could be classified as escape strategies (e.g., earlier flowering) and drought tolerance (e.g., transpiration rate, osmoprotectant content and root-to-shoot ratio) (Meyre et al., 2001). Studies of physiological adaptive changes to drought suggest that phy modulates water stress responses since a former study using *Nicotiana tabacum* mutants deficient in phy chromophores (Kraepiel et al., 1994) and later studies using Arabidopsis *phyB* mutants (Boccalandro et al., 2009; Boggs et al., 2010) showed changes in the control of leaf transpiration. In addition, leaf water loss is highly correlated with stomatal conductance and its negative regulator abscisic acid (ABA) (Kriedemann et al., 1972). In some species, studies have corroborated the hypothesis that stomatal conductance is

negatively regulated by phyB in drought stress conditions. For example, in Arabidopsis (Boggs et al., 2010; González et al., 2012) and tomato (D'Amico-Damião et al., 2015), *phyB* mutants (*phyB1* and *phyB2* in tomato) presented lower stomatal conductance than the wild-type (WT) in drought stress conditions and this may be related to the induction of ABA synthesis in Arabidopsis (Boggs et al., 2010; González et al., 2012). However, González et al. (2012) attributed the lower drought tolerance of Arabidopsis *phyB* mutants to lower ABA sensitivity, instead of ABA content, as the WT and *phyB* mutants showed similar ABA levels. Moreover, the reduction in expression of ABA-signalling genes, such as *ABCG22* (*ATP-binding cassette*) (Kuromori et al., 2011) and *PYL5* (family of *pyrabactin resistance*) (Santiago et al., 2009; Park et al., 2009), in *phyB* mutants (González et al., 2012) likely influence the lower sensitivity of *phyB* mutants to ABA.

Taken together, these reports show that phyB plays an important role in droght stress tolerance and in the avoidance of water-loss via stomatal closure. However, stomatal closure followed by decreased conductance may also reduce the photosynthetic rate, as shown by Boccalandro et al. (2009). These authors observed that Arabidopsis *phyB* mutants exhibited lower stomata density, resulting in a lower transpiration rate that decreased the CO_2 uptake. Although drought-tolerance improvement via the reduction of water-loss seems positive, the lower CO_2 uptake may be dangerous for plants under high photosynthetic active radiation exposure. For example, the excess of excitation energy not used in the photochemistry step of the photosynthesis reaction (because of low CO_2 concentrations) may be transferred to molecular oxygen, synthesizing harmful reactive oxygen species (ROS) if not sufficiently dissipated by the fluorescence or heat (Niyogi, 1999). These studies suggest that *phyB* mutants would be susceptible to drought stress. However, other studies showed that phyB was part of other signalling pathway in the drought stress response, such as the antioxidant system. Indeed, ascorbate peroxidases (APXs) and catalases (CATs) were upregulated in rice *phyB* mutant genotypes in drought stress conditions compared to the WT, and this upregulation had a positive effect on the *phyB* mutant plants, which acquired a drought-tolerance phenotype (Yoo et al., 2017).

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These elucidations may contribute to the maintenance of grain yield in rice *phyB* mutants, even with a decreased photosynthesis rate. Liu et al. (2012) found a lower stomatal density and net CO_2 uptake with no effect on rice production in *phyB* mutant drought-stressed plants. phyB may also modulate drought tolerance in tomato plants, specifically the *phyB1* mutant. Gavassi et al. (2017) exposed 7-day-old seedlings of tomato to a low water potential solution and detected higher shoot and root lengths in *phyB1* mutants compared to WT, as well as higher content of proline and glycine-betaine. Proline and glycine-betaine are helpful osmoprotectants that actively support the osmotic regulation of cells, which helps plants cope with drought injury (Abro et al., 2019). The aforementioned escape strategy also contributes to give importance to phyB as drought stress mediator. Rice *phyB* mutants exhibited earlier flowering than the WT after water-deficit treatment and exhibited the two mechanisms of perception, both associated with flowering-related gene expression, but some were ABA-dependent and others were ABA-independent (Du et al., 2018). Taken together, the majority of data suggests that phyB is a negative regulator of drought tolerance acting on different mechanisms (Table 1), such as the antioxidant system, accumulation of osmoprotectants, stomatal movement, regulation of phytohormones and expression of stress-related genes.

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Table 1 – Summarized data of characteristic phyB-regulated in salt stress, drought stress and low temperature stress.

SALT STRESS				
Plant specie	**Treatment**	**Type of phyB**	**Effect of phyB on plant (phyB1 in tomato).**	**References**
Nicotiana tabacum	800 Mm NaCl	phyB	Increase: MDA, eletrolyte leakage. Reduce: Chlorophyll, proline, antioxidant activity, Net photosynthesis rate, ABA content, JA content.	Yang et al. (2018)
Tomato	100 nM NaCl	phyB1 and phyB2	Increase: MDA. Reduce: root lenght, chlorophyll, carotenoids and proline	Gavassi et al. (2017)
	100 nM NaCl	phyB1	Increase: MDA, H_2O_2. Reduce: Chlorophyll, proline, antioxidant activity, Net photosynthesis rate.	Cao et al. (2018)
Rice	200 Mm NaCl	phyB	Increase: Na^+ content, Na^+/K^+ ratio. Reduce: Chlorophyll, *OsHTKs* expression, Fresh weight, survival rate.	Kwon et al. (2018)

DROUGHT STRESS				
Arabidopsis	4-6% volumetric water content	phyB	Increase: stomatal conductance Reduce: ABA concent.	Boggs et al. (2010)
Arabidopsis	7 days without watering	phyB	Increase: stomatal conductance, *ABCG22* and *PYL5* expression, drought tolerance phenotype.	González et al. (2012)
Tomato	5 days without watering	phyB	Increase: stomatal conductance.	D'Amico-Damião et al. (2015)
Tomato	PEG at Ψw of −0.3 MPa	phyB1 and phyB2	Increase: MDA. Reduce: shoot lenght, Root lenght, chlorophyll, carotenoids, proline, glycine-betaine.	Gavassi et al. (2017)
Rice	16 days without watering	phyB	Increase: leaf area, stomatal density, transpiration rate, Net CO_2 uptake. Reduce: proline content, recovery plants.	Liu et al. (2012)
Rice	4 days without watering	phyB	Increase: H_2O_2. Reduce: antioxidant activity.	Yoo et al. (2017)
Rice	10-20% soil water content	phyB	Increase: days to flowering.	Du et al. (2018)

LOW TEMPERATURE STRESS				
Arabidopsis	Cold (-9°C) for 1h or cold (-5°C) for 0,5h	phyB	Increase: survival rate, *CBF* expression, *COR* expression. Reduce: ion leakage	Jiang et al. (2020)
Tomato	Cold (4°C) for 7 d	phyB	Increase: eletrolyte leakage. Reduce: survival rate, *CBF1* expression, *COR* expression, ABA, JA.	Wang et al. (2016)
Rice	Cold (4°C) for 4 d	phyB	Increase: MDA, eletrolyte leakage. Reduce: survival rate, *CBF/DREB1s* expression.	He et al. (2016)
Rice	Cold (4°C) for 24 h	phyB	Increase: photoinibition. Reduce: USFA, chlorophyll.	Yang et al. (2013)

2.2. Salt stress

Soil salinity is an increasing problem in agriculture, which drastically affects plants growth (Machanda and Garg, 2008). Many efforts have been made to alleviate salinity conditions through the improvement of soil quality (Hasini et al., 2020). However, physiological alteration in plants is also a potential tool to improve tolerance in these environmental conditions because salt stress activates complex downstream signalling pathways (Holm et a., 2001; Trifunović-Momčilov et al., 2020; Wani et al., 2020). These pathways are primarily related to the increase in ROS, which is associated with oxidative damage in salt-stressed plants (Akyol et al., 2020). Various studies have shown that ROS (e.g., hydrogen peroxide and superoxide) are increased in plant tissue during salt stress, as well as upregulation of antioxidant ezymes under longer stressors conditions (e.g., CAT, APX, superoxide dismutase [SOD], glutathione reductase, guaiacol peroxidase [GPOX] and peroxidase [POD]) (Asrar et al., 2020; Cheng et al., 2020; Fadzilla et al., 1997; Hossain and Dietz, 2016; Li et al., 2017). Therefore, ROS and antioxidant enzymes are indicators of plant stress acclimation. Higher concentration of ROS may lead to cell damage via lipid peroxidation in cellular membranes (Kwiecien et al., 2014), which is a critical reason for plants to avoid high increments of these compounds. Plants exhibit protective mechanisms for survival under salt stress conditions via the synthesis of ROS scavengers. Many ROS scavengers have already been described in plants (Mittler, 2002). Other compounds, synthesized via the conversion of heme catalyzed by heme oxygenase (HO) (Mahawar and Shekhawat, 2017; Tenhunen et al., 1968), have similar antioxidant functions, such as biliverdin (BV), which also acts as a radical trap (Stocker et al., 1987). For example, the activity of GPOX and SOD were increased in soybeans leaves after BV pretreatment in salt-stressed plants (Balestrasse et al., 2008).

Evidence indicates that HO in controlled by phy, since a phytochrome chromophore-deficient mutant (*pcd1*) of *Pisum sativum* plants lacked HO activity (Weller et al., 1996). Certainly, these results suggests that phy play a role in the regulation of ROS scavengers through HO activity (e.g., GPOX and SOD), but which phy is responsible for these effects is unknown. Additionally, reciprocal regulation between phy and HO was observed since and Arabidopsis mutant, deficient in HO (*hy1*

mutant), was completely insensitive to R/FR and exhibit long hypocotyl in white light (Terry et al., 2002). Such circumstances entail the cooperative influence of phy and HO to induce salt stress tolerance through antioxidant system enhancement.

In addition to the tight and complex relationship between ROS scavengers (HO) and phy synthesis in salt-stressed plants, phy-mediated light signalling could also alter the transcription of proteins that were previously described to confer salt tolerance in plants. For example, the protein salt tolerance-related (STO) improved root growth in Arabidopsis transgenic plants overexpressing *STO* under salt stress (Nagaoka and Takano, 2003). Thus, phyB likely interacts with *STO* since *STO* was described to be repressed by COP1 (Indorf et al., 2007) and the SPA1-COP1-PIF1 (PIF1) kinase regulatory complex, which is negatively regulated by phyB (Paik et al., 2019). In other words, phyB repressed the SPA-COP1-PIF1 complex, allowing transcription of STO, which in turn induced root growth under salt stress conditions (Figure 1).

Recent data corroborated the role of phyB in salt stress regulation but as negative regulator. For example, seedlings of tomato *phyB1* mutants showed lower oxidative damage after stress treatment, as demonstrated by the reduction of malondialdehyde (MDA) content (Gavassi et al., 2017). Similarly, Cao et al. (2018) manipulated light quality to increase inactive phyB1-form content, which mimics the behavior of *phyB1* mutants; these authors observed that the plants were salt-tolerant after exposure to low R:FR ratios with improvement of many desirable traits, such as increased ROS scavenger activity (CAT, POD and SOD) and decreased ROS content (superoxide and hydrogen peroxide).

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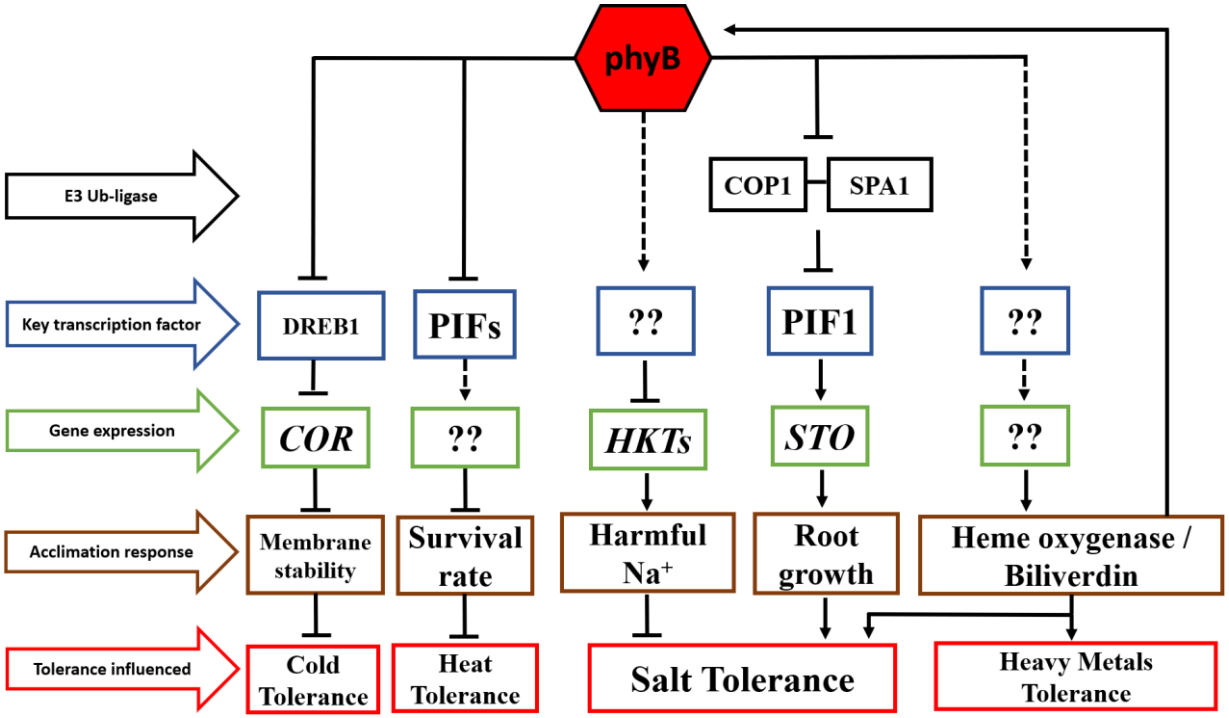

Figure 1. Schematic of the role of phyB in the acclimation responses to cold, heat, salt and heavy metal stress. From the red-light signalling, phyB coordinates the negative regulation of COP1-SPA-dependent degradation of PIF1 transcription factors, which triggers changes in stress response target genes expression. phyB coordinates other TIFs with no reported evidence of a role for the COP1-SPA complex. Note that these are key pathways in the induction of genes related to signalling and/or the biosynthesis of ROS scavengers and stress components. Although the complex signalling of phyB in stress acclimation involves some unknown components, this photoreceptor plays a crucial role in red-dependent stress responses. Arrows indicate upregulation, T-bars indicate down-regulation, and dotted lines indicate unknown (?) signalling routes. COP1, constitutive photomorphogenic 1; *COR*, *cold-responsive* gene; DREB1, dehydration-responsive element binding protein 1; *HKTs*, *high-affinity* K^+ *transporters* genes; PIF1, phytochrome interacting factor 1; PIFs, phytochrome interacting factors; SPA1, suppressor of PHYA-105; STO, *salt tolerance-related* gene.

phyB and its complexity in salt tolerance improvement is not limited to oxidative stress and related compounds. Other proteins with different modes of action were described as salt tolerance-inductors, which were also mediated by phyB. For example, the transporter high-affinity K^+ transporters (HKTs) played a pivotal role in avoiding the accumulation of harmul Na^+ concentration and enhanced salinity tolerance (Deinlein et al., 2014). In rice, phyB repressed the expression of putative *OsHKTs* gene, a convenient regulation that was effective on plant salt-tolerance induction, considering the lower content of Na^+ found in rice *phyB* mutants (Kwon et al., 2018). Therefore, these perspectives open a new avenue for up-stream activity of phyB family in salt stress response. Similar to other abiotic stresses, the relationship between adaptative salt stress mechanisms and phytohormonal dynamics must not be overlooked, as ABA and JA are correlated with salt stress alleviation in plants (Kang et al., 2005; Khan et al., 2019; Zhang et al., 2006; Zhu et al., 2019). The exogenous application of both phytohormones to strawberry plants (*Fragaria* x *ananasa* Duch.) activated protective mechanisms against salt stress, such as antioxidant capacity and phenolic compounds (Jamalian et al., 2020) Nevertheless, the phytohormonal influence on salt stress tolerance adjustments suggests that phyB plays a role in salt tolerance via phytohormonal modulation (ABA and JA). For instance, salt stress treatment increased the ABA and JA content in *phyB* mutants of *Nicotiana tabacum*, which ameliorated many salt-tolerance characteristics, decreasing MDA content and electrolyte leakage, while increasing the antioxidant system (Yang et al., 2018). Although phyB-regulated responses differed in past studies as a positive or negative regulator of salt stress tolerance, most current evidence highlights that phyB is a negative regulator of salt stress (Table 1). However, many defence-associated genes were up-regulated in responses to salt stress in *Nicotiana tabaum phyB* mutants, such as *NtLEA5* (*late embryogenesis abundant protein*), *NtER10C* (*early responsive dehydration*) and *NtABF2* (*ABA-responsive element binding*) (Grover et al., 2001; Yang et al., 2018). Thus, further studies are required to identify the phyB-regulated mechanisms that affect acclimation responses to salinity.

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2.3. Low and high-temperature stress

Temperature is an environmental factor that affects crucial responses during plant growth, from seed germination to flowering. Plants naturally display strategies to perceive environmental temperature fluctuations and then trigger seed germination, which could guarantee ideal conditions for seedlings to rise. For example, low/high temperatures could break seed dormancy (Kozlowski and Pallardy, 2002) and interestingly, phyB contributes to breaking cold-induced dormancy (Donohue et al., 2008). In addition to seed responses to temperature stress, heat and cold tolerance were described as a great evolutionary characteristic that allowed plants to thrive along the Earth ages. Notably, plants show different responses to heat and cold stress based on the incident light quality, influencing fundamental characteristics for cell survival. The stability and fluidity of cell membranes is a crucial aspect in temperature acclimation responses, the main target of which is the thylakoid membrane, which upon cold stress can suffer damage (Yordanov, 1992), leading to electron leakage from the photochemical reaction and ROS formation (Niyogi, 1999). Composition of the plasma membrane in terms of the higher unsaturated fatty acid (USFA) to saturated fatty acid (SFA) ratio (USFA:SFA ratio) was closely correlated with the stability and fluidity of cell membranes and, thus, to chilling tolerance (Ishizaki et al., 1996; Szalontai et al., 2003). Indicators, such as higher electrolyte leakage (Murray et al., 1989) and increased MDA content (Jouve et al., 1993), are largely used to identify injured cell membranes.

In a pioneering work, Williams et al. (1972) demonstrated that a night break with R suppressed cold acclimation in *Cornus stolonifera*, as shown by higher leaching of the electrolyte content. However, the suppression was relieved when the R was followed by FR. Although this work did not identify the main photoreceptors involved, the results suggest that phytochromes were part of the cold stress response. In fact, phytochromes have also been shown to be thermosensor pigments, specially phyB, as demonstrated by Legris et al. (2016), who proposed that the main mechanism underlying phyB responses was the quick reversion to Pr triggered by temperature in a light independent-manner. For example, rice plants lacking phyB (*phyB* mutants) showed increased cold tolerance verified by lower electrolyte leakage and higher

seedling survival rate compared to WT plants (He et al., 2016). This may be assigned to higher stability of USFA synthesis that also alleviated the photoinhibition caused by chilling (Yang et al., 2013).

However, contradictory data have also been registered, in which after freezing treatment, Arabidopsis *phyB* mutants and phyB-overexpression presented higher and lower electrolyte leakage, respectively (Jiang et al., 2020). Cold tolerance positively regulated by phyB seemed to involve a complex molecular mechanism, especially those related with the expression of cold-related (*COR*) genes, such a*s cor14b* (Crosatti et al., 1999) and *cor15a* (Kim et al., 2002). In Arabidopsis, expression of the latter cold-related genes was activated by transcription factors C repeat binding factors and drought response element binding factor 1 (CBFs/DREB1s) that were phyB-induced during cold exposure (Kim et al., 2002). Therefore, downstream action of the CBFs/DREB1s transcriptional factor was suggested in cold acclimation responses since He et al. (2016) reported that cold tolerance in rice was negatively regulated by phyB, demonstrating that CBFs/DREB1s was negatively regulated by phyB, conferring a cold resistant phenotype in rice phyB mutants.

However, the underlying mechanisms of chilling tolerance mediated by phyB are complex and involve other key molecules that comprise a huge number of pathway cascades that may include phytohormone regulation. Similar to rice plants, lack of phyB in tomato (mutants *phyB1* and *phyB2*) increased the expression of *COR* genes, but it was suggested to be dependent on ABA and JA, since these hormones act to up-regulate the transcript levels of *COR* genes. Additionally, the levels of ABA and JA were increased in tomato *phyB1 a*nd *phyB2* mutants after cold treatment (Wang et al., 2016).

Temperature stress regulated by phyB is not limited to lower temperatures and also extends to heat stress. Gavassi et al. (2017) reported longer shoot length in *phyB1* mutants compared to the WT after heat stress exposure. This mutant also presented lower MDA content (a product of lipid peroxidation), suggesting a presumptive phyB role in alleviating the deleterious effect of ROS. Notably, phyB signalling of heat stress may occur via the negative control of putative basic helix–loop–helix transcription

factors, such as PIFs (Leivar et al., 2012; Lorrain et al., 2008; Martínez et al., 2018). Among the known PIFs, phytochrome interacting factors 4 and 5 (PIF4 and PIF5) (Koini et al., 2009; Stavang et al., 2009), as well as phytochrome interacting factor 7 (PIF7) (Fiorucci et al., 2020), have been shown to have a crucial role in the hypocotyl elongation of Arabidopsis during high temperature stress. Qiu et al. (2019), demonstrated a substantial reduction of hypocotyl growth in Arabidopsis *pif4-2* mutants compared to the WT; the authors clearly showed that the thermo-sensing responses to heat were more pronounced under R light and LD conditions, which suggests the role of phyB in thermosensory machinery to warm conditions. Such evidence was also supported by Song et al. (2017) in Arabidopsis *phyB* mutant seedlings that exhibited a higher survival rate and lateral root length after heat stress treatment compared to WT seedlings. These responses, shown by *phyB* mutants, were directly related to an increase in PIF accumulation, since Arico et al. (2019) demonstrated that the thermotolerance acquired by Arabidopsis *phyB* mutants (lower proportion of damaged plants after heat shock) was not pronounced in *pifq* mutants (*pif1*, *pif3*, *pif4* and *pif5*), which exhibited no difference from WT plants. In contrast to cold stressed plants, heat stressed plants decreased the levels of USFA, which can reduce the targets of oxidative damage (double bonds between C-atoms) and increase the thermotolerance to heat stress (Das and Roychoudhury, 2014). In addition, higher thermotolerance could be attributed to lower USFAs in *phyB* mutants (Arico et al., 2019). As discussed above, the TFs involved in heat stress acclimation are apparently distinct from the TFs involved in cold stress acclimation (Figure 1), which may explain how the same photoreceptors help plants to cope with two antagonistic stresses.

2.4. High light stress

Light is one of the most important factors for plants, controlling growth through photosynthesis and photomorphogenesis processes (Carvalho et al., 2011b). However, light could also be a stressor agent when its quantity is higher than photosynthetic capacity. High light provides an excess of excitation energy that leads to electron leakage from photochemical reactions and the electron transport system, which induces ROS generation and cell damage (Kimura et al., 2003; Niyogi, 1999). To cope with the deleterious effects of an excess of light, plants have established

several mechanisms, including increased activity of ROS scavenger, accumulation of accessory pigments, and anthocyanins to decrease light damage in shoot tissues (Asada, 1996; Gould et al., 2010). Interestingly, phytochrome involvement in alleviating high light stress has been suggested because of the negative regulation of chlorophyll (component of antenna complex) biosynthesis by PIF1, since chlorophyll accumulation was increased in Arabidopsis *pif1* mutants and decreased in *phyB* mutants (Huq et al., 2004). Additionally, PIF4 and PIF5 were reported to negatively regulate anthocyanin biosynthesis under R (Liu et al., 2015), as these results attributed a positive role to phyB in high light stress tolerance (in terms of intensity), preventing the formation of ROS that would be increased if these pigments (chlorophyll and anthocyanin) were suppressed. Accordingly, phyB1 and phyB2 photoreceptors showed an important function during tomato plant exposition to high light stress; the *phyB1phyB2* double mutant had a strong decrease in quantum yield (Fv/fm) and photosynthesis rate compared to WT plants, in addition to pigment (chlorophyll, carotenoids and anthocyanins) reduction in the *phyB2* mutant after 2 hr of exposure to high-intensity light (900 μmol m^{-2} s^{-1} of white light) (Kreslavski et al., 2020).

High light stress is also related to the light quality that comprise the short-wavelengths bands of the electromagnetic spectrum, such as UV-C (220–280 nm), UV-B (280–320 nm) and UV-A (320–400 nm) (Frederick et al., 1989). These short-wavelengths or UV have claimed scientific attention over the years, given the current scenarios of climate change and ozone layer damage that allow higher proportions of hazardous radiation to reach the earth's surface (Bernhard et al., 2020). Plants suffer serious injuries when exposed to a high proportion of UV, which limits their production potential as a result of the negative effect on important physiological mechanisms, such as the photosynthetic apparatus (PA), which includes damage to photosystem II (PSII), lower ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) regeneration, low Fv/fm and reduced photosynthetic pigment accumulation (Teramura and Sullivan, 1994). Therefore, two mechanisms are used to cope with the detrimental effects of high UV proportions: (a) enhanced repair mechanisms and (b) UV screening pigment synthesis. The enhancement of repair mechanisms included UV-damaged DNA restoration upon light-dependent photoreactivation by photolyases and

acclimation responses (Britt, 1996), including an increase in ROS scavengers (Rao et al., 1996). An increase in the synthesis of UV screening pigments includes flavonoids and carotenoids (Cen and Bornman, 1990). These physiological alterations are the most studied and reported responses, with huge potential to improve the UV stress-relieving mechanisms.

Research showed an interaction between UV-B and R light wavelengths in the induction of cotyledon opening; exposure of UV enhanced the phyB perception of R light radiation and *phyB* mutants had impaired cotyledon opening under UV-B and R (Boccalandro et al., 2001). Therefore, a possible interaction between phyB and a specific UV photoreceptor could be suggested; for example, UVR8 interacted with the central regulator of light signalling COP1, since both proteins accumulate in the nucleus under UV exposure in Arabidopsis (Rizzini et al., 2011), which resulted in a reduction in hypocotyl length (Favory et al., 2009). Moreover, UVR8 activity in the promotion of UV tolerance was largely reported in different species, mainly through induction of flavonoids and anthocyanin synthesis in Arabidopsis (Kliebenstein et al., 2002; Favory et al., 2009) and tomato (Liu et al., 2020). In addition, both UVR8 and phyB regulate COP1 activity. Gao et al. (2018) showed that *phyB* mutants of Arabidopsis grown under UV-B light increased *UVR8* photoreceptor gene expression. In other words, phyB likely negatively regulates UV-tolerance through suppression of *UVR8* genes, which are known promotors of UV-tolerance (Figure 2). In fact, the evidence that UV-tolerance is negatively regulated by phyB has also been reported in tomato, but not on a molecular level. For example, *phyB1* and *phyB2* mutants were more tolerant to UV-B exposure and showed higher shoot and root dry weight and shoot and root length, as well as a decreased MDA content (Gavassi et al., 2017). However, these insights are preliminary and more studies are required to elucidate the crosstalk mechanisms between phyB and URV8, considering that *phyB1* and *phyB2* mutants also exhibited lower anthocyanin content and anthocyanin accumulation that is an UV-tolerance characteristic, contradicting the positive role of phyB.

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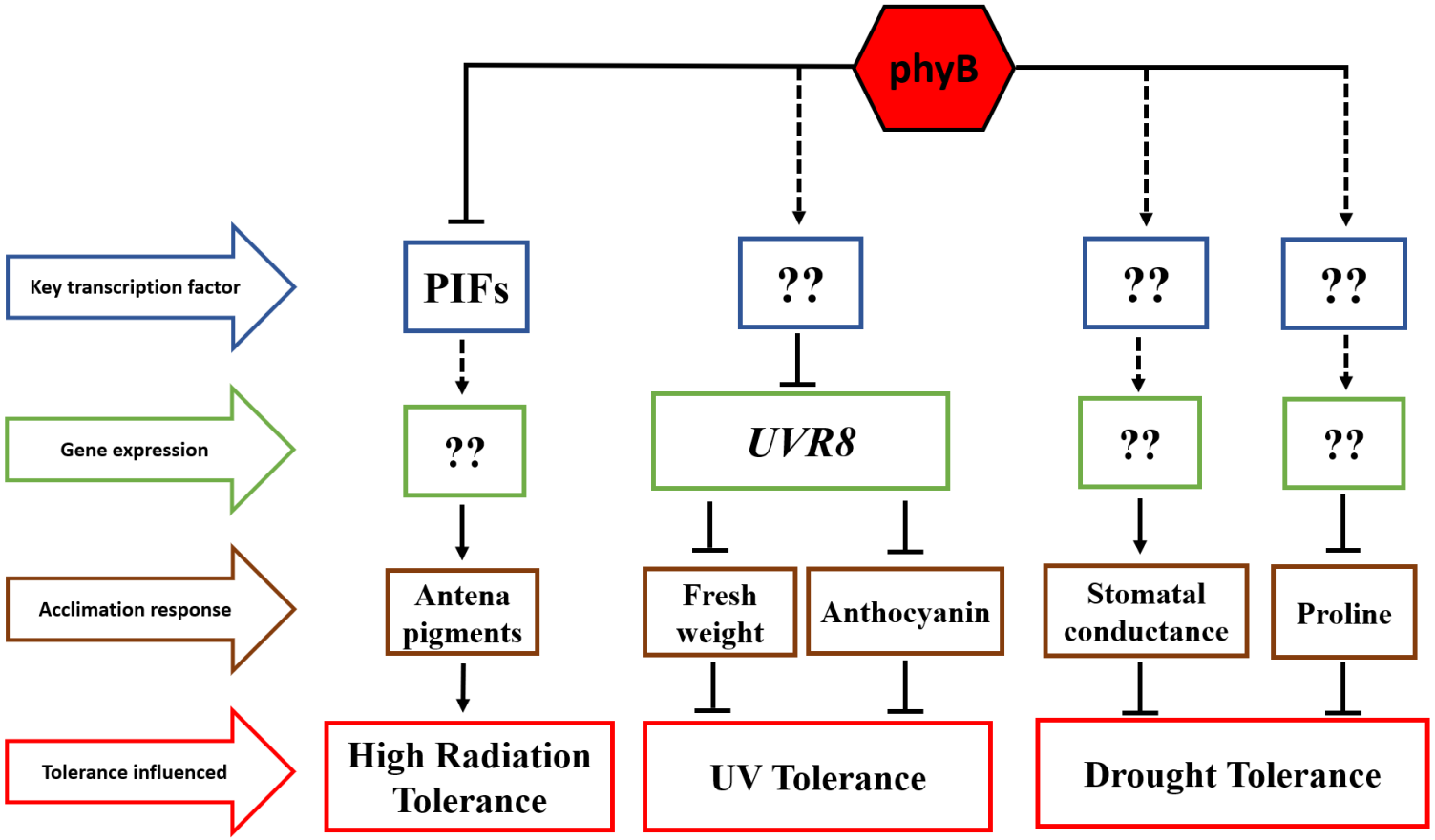

Figure 2. Schematic of the role of phyB in acclimation responses to high radiation (intensity), UV and drought stress. From the red-light signalling, phyB coordinates the regulation of unknown TIFs and triggers changes in some stress response gene expression. Note that these are key pathways in the induction of genes related to signalling and/or the biosynthesis of components related to acclimation response. Although the complex signalling of phyB in stress acclimation involves some unknown components, this photoreceptor plays a crucial role in red-dependent stress responses. Arrows indicate upregulation, T-bars indicate down-regulation, and dotted lines indicate unknown (?) signalling routes. PIFs, phytochrome interacting factors; UVR8, *UV-resistance locus 8*.

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Whether phys act as negative or positive regulators of UV tolerance is not well established (Table 2). Other findings also showed that phytochromes are positive regulators of UV-tolerance since Arabidopsis *phyAphyB* double mutants had lower PA resistance and a decreased photosynthesis rate after UV-B exposure (Kreslavski et al., 2017). However, positive or negative regulation of UV stress tolerance by phyB seems to be species specific, since *PHYB-ovx* plants of potato (*Solanum tuberosum* – tolerant) and Arabidopsis (susceptible) showed opposite responses for Fv/fm and pigment content (both increased in potato and decreased in Arabidopsis) during exposure to UV-B light in potato and UV-A light in Arabidopsis (Kreslavski et al., 2015, 2016).

phyB also acts on other UV light tolerance strategies. For example, programmed cell death (PCD) is a mechanism that maintains tissue homeostasis during plant growth under stress condition and enables nutrient remobilization from dying cells (Nawkar et al., 2013). Exposure to UV can trigger ROS generation, which can damage cells, resulting in PCD (De Pinto et al., 2011). Under UV, phyB was described to regulate PCD through unknown mechanisms as Arabidopsis *phyB* mutants showed a reduction in ROS and ROS scavenger (SOD, CAT and APX) compared to the WT plants under UV-C, but did not restrain PDC progression (Rusaczonek et al., 2015). Thus, phyB-dependent ROS production under UV is not a key factor involved in PCD, and a multitude of phyB-dependent TIFs may be associated with the response to UV stress (Chen et al., 2013), suggesting that further studies are required on UV stress to unravel the involvement of other phyB-regulated mechanisms.

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Table 2. Summarized data of characteristics phyB-regulated in high temperature stress, high light stress, and heavy metal stress.

Plant species	Treatment	Type of phyB	Response on plant	Reference
HIGH TEMPERATURE STRESS				
Arabidopsis	Heat (37°C) for 3 d	phyB	Reduce: survival rate, lateral roots length.	Song et al. (2017)
Arabidopsis	Heat (45°C) for 45 min	phyB	Increase: electrolyte leakage and USFA.	Arico et al. (2019)
			Reduce: plants without damage (%), Plant survival (%), SFA	
Tomato	Heat (42°C) for 6h d^{-1}, during 3 d	phyB1 and phyB2	Increase: anthocyanin and MDA.	Gavassi et al. (2017)
HIGH-LIGHT STRESS				
Arabidopsis	Rc (10 $molm^{-2}s^{-1}$)	phyB	Increase: anthocyanin content.	Liu et al. (2015)
Arabidopsis	UV-B (10.08 kJ m^{-2}) for 8 h d^{-1}, during 15 d	phyB	Reduce: *UVR8* expression, hypocotyls length, petiole length.	Gao et al. (2018)
Arabidopsis	UV-A (12 W m^{-2})	phyB	Increase: PA resistance, photosynthesis rate.	Kreslavski et al. (2017)
Arabidopsis	UV-A (10 W m^{-2})	phyB	Increase: Fv/Fm, chlorophyll, carotenoids.	Kreslavski et al. (2016)
Arabidopsis	UV-C (200 mJ cm^{-2})	phyB	Increase: ROS, antioxidant activity	Rusaczonek et al. (2015)

Tomato	WL (900 µmol m^{-2} s^{-1})	phyB1 and phyB2	Increase: Fv/Fm, Photosynthesis rate.	Kreslavski et al. (2020)
		phyB2	Increase: chlorophyll, carotenoids, anthocyanin.	
Tomato	UV-B for 8h d^{-1}, during 3 d	phyB1 and phyB2	Increase: anthocyanin and MDA.	Gavassi et al. (2017)
			Reduce: shoot dry weight, root dry weight, shoot length, root length, chlorophyll, carotenoids.	
Potato	UV-B (12 W m^{-2}) for 45 min	phyB	Increase: Fv/Fm, chlorophyll, carotenoids, UV-absorbing substances.	Kreslavski et al. (2015)
HEAVY METAL STRESS				
Tomato	65 mM $CdCl_2$	phyB1 and PhyB2	Increase: MDA.	Gavassi et al. (2017)
			Reduce: shoot length, Shoot dry weight, carotenoids (only phyB1).	

2.5. Heavy metal stress

Heavy metal (HM) contamination in soil is one of the main topics in the debate about food security and food safety worldwide (Kong, 2014; Tóth et al., 2016). Although agriculture has produced food for the growing population demand in a more sustainable way, the high use of inorganic fertilisers has contributed to the accumulation of HMs in the soil (Atafar et al., 2010; Wang et al., 2020). In fact, fertilisers are contaminated by HMs (Gimeno-García et al., 1995; Mortvedt, 1996; Wang et al., 2020) and their use could affect crop yield by high HM accumulation in the soil. Plant exposure to HMs results in toxicity, represented by rapid growth inhibition and decreased PA activity (Skórzyńska-Polit and Baszyński, 1997; Alaoui-Sossé et al., 2004), related to oxidative stress induced by HM (Mithöfer et al., 2004). Therefore, a strategy that could mitigate the deleterious effects of unavoidable HM toxicity in cash crops reaches physiological alterations that comprises the phytochrome role.

On a molecular level, evidence has shown that As (arsenic) treatment (120 μM of sodium arsenate - Na_2HAsO_4) in Arabidopsis down-regulated the expression of *PIF3*, a known component in the phytochrome signalling pathway (Shukla et al., 2018). Despite being elusive, such influences indicate that phytochromes may also have a part in the signalling pathways of HM stress acclimation. Gururani et al. (2016) studied the influence of a high level of Zn (20 nM $ZnCl_2$) and reported that *Agrostis stolonifera* L. transgenic lines (S599A-14 and S5994-18 – hyperactive of phyA) exhibited lower hydrogen peroxide (H_2O_2) content and increased proline content, antioxidant activity, and plant dry weight compared to the WT. Moreover, this was likely associated with phytochrome positive regulation of ROS-scavenger via HO activity (discussed in salt stress topic), which was also reported to alleviate HM stress in *Medicago sativa* (Cui et al., 2012). Conversely, phyB-mediated HM tolerance seems to vary among species or between developmental growth stages. In 7-day-old *phyB1* and *phyB2* mutant tomato seedlings, Gavassi et al. (2017) reported higher dry weight and lower MDA than the WT after cadmium (Cd) treatment (65 mM of $CdCl_2$). However, both tomato mutants did not show any changes compared to the WT in 21-day-old plants under Cd treatment (150 mM of $CdCl_2$) (Gaion et al., 2018). However, further studies are needed

to elucidate whether phyB influences HM stress responses, considering the lack of reports about this topic.

2.6. Epilogue

This paper is an overview of how phyB regulates various environmental stresses that negatively affect plant development under these adverse growth conditions. The well-known role of phyB as a negative regulator of photomorphogenesis reveals characteristics of interest for improving plant tolerance to both abiotic and biotic stresses, mainly through phyB-mediated physiological and/or morphological changes in stressed plants. However, the stress responses regulated by phyB seem to be complex, since phyB could modulate the characteristics of tolerance to different stresses, positively or negatively, and depending on the plant species studied. The majority of data suggests that for increasing tolerance to drought, salinity, high temperature, and low temperature the suppression of phyB-signalling seems to be beneficial, whereas to increase tolerance to heavy metals, induction of phyB-signalling is required. However, no pattern between species was observed for phyB-mediated high light and UV stress tolerance characteristics. Thus, a meticulous assessment of a wide range of plant species is necessary to better understand the underlying phyB-dependent mechanisms that affect high light and UV stress responses in plants. Taken together with world climate changes, these results suggest the importance of the development of plants that are tolerant to the most diverse environmental stresses and that phyB photoreceptor may be yet another target molecule for plant breeding. Thus, manipulation of the phyB family (overexpression or knockout) resulted in traits of agronomic interest for the improvement of plant tolerance to environmental stress, but more comprehensive studies on different plant species, stages of plant development, and combinations of various stressors are important to show the consistency of phyB-mediated responses during biotic and abiotic stress.

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CHAPTER 2 - Phytochrome B1 of tomato (*Solanum lycopersicum* L.) modulates responses to drought stress in vegetative and reproductive stage growth

ABSTRACT – Physiologically plants have many strategies to cope with drought stress condition, for example, evidence has shown that a photoreceptor that perceives red light, phytochrome B1, is part of responses to drought stress. However, is not known whether such responses are carried until the end of plant cycle, influencing yield and quality of fruit. For this purpose, the experimental design consisted of factorial entirely randomized with two factors – genotype and irrigation treatment. Thus, the mutants phytochrome B1 deficient (*phyB1*) and its isogenic line wild-type (WT) were cultivated in a greenhouse under two reposition of daily water evapotranspiration (ETP): 100% and 50% ETP, performed in two moments: (I) in vegetative stage growth – starting at 26 days after sowing (DAS) and ending at 36 DAS; (II) in reproductive stage growth – starting at 33 DAS and ending at 41 DAS. At the end of ETP treatments were performed analysis of dry weight of leaf, stem, root as well as leaf area, stem diameter/length and root length. After the last harvest (135 DAS) were evaluated fresh and dry weight of fruits, diameters and soluble solid (°Brix). When water deficit occurred in the vegetative stage growth, *phyB1* presented higher vegetative biomass accumulation than WT, but WT presented higher root development and fruit yield. In the other hand, *phyB1* showed lower fruit size and higher °Brix in fruit when water deficit was performed in reproductive stage growth. Therefore, to raise tomato plants that keep their vegetative growth in water shortage without concern about fruit yield, overexpression of phyB1 would be advantageous. In the opposite manner, if the priority is fruit quality, phyB1 knockout would be beneficial.

Keywords: Biomass accumulation, drought tolerance, tomato yield, Source-sink partitioning, Evapotranspiration, °Brix.

1. INTRODUCTION

The light controls the development of plants through a complex system of photoreceptors, which participate in almost all responses from germination to flowering. For example, cryptochromes and phototropins, mainly detect the wavelengths of blue light (320–400 nm), and phytochromes, present absorption peaks in the red (V; ~ 660 nm) and extreme red (VE; ~ 730 nm) range of the light spectrum (Sancar, 2003; Carvalho et al., 2011). However, phytochromes are the most characterized and exponentially studied. These pigments are dimeric proteins (~130 KDa) covalently linked to a phytocromobilin, a linear open-chain tetrapyrrole that acts as a chromophore. Evidence that angiosperms have several species of phytochromes, encoded by a small family of genes, was initially verified in studies with *Arabidopsis thaliana*. Five phytochrome genes have been isolated in this species: *PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE*, which code for the apoproteins PHYA, PHYB, PHYC, PHYD and PHYE (Sharrock and Quail, 1989). Such apoproteins, after binding to the chromophore, form the phytochromes phyA, phyB, phyC, phyD and phyE. Currently, the molecular characterization of phytochromes has been carried out for several species, including tomato (*Solanum lycopersicum* L.), one of the most important vegetables in the world. In this species, five genes for apoproteins were also found: PHYA, PHYB1, PHYB2, PHYE and PHYF (Pratt et al., 1997).

Since the discovery of phytochromes, studies in several species have revealed important functions of these photoreceptors during plant development, such as seed germination (Dechaine et al., 2009; Oh et al., 2009), flowering (Andres et al., 2009; Brock et al., 2010), hypocotyl elongation (Yang et al., 2009; Kunihiro et al., 2010) and flavonoid and carotenoid synthesis (Carvalho et al., 2010; Toledo-Ortiz et al., 2010). Furthermore, recent discoveries have revealed an important role of phytochromes in modulating responses to biotic and abiotic stresses. For example, it has been shown that phytochromes are part of the signalling responses to herbivory (Howe & Jander, 2008; Ballaré, 2009), low or high temperatures (Donohue et al., 2008; Foreman et al., 2011), excess harmful radiation (eg. Ultra-Violet-B or UV-B) (Boccalandro et al., 2001), salinity (Balestrasse et al., 2008; Datta et al., 2008) and more elusively, heavy metals (Cui et al., 2011; Gaion et al., 2018; Gavassi et al., 2017).

However, it was brought to attention the fact that phytochromes are part of the responses to stress induced by water deficit, the most worrying stressor for agronomic cultivation. For example, during water deficit, the *pew1* mutant of *Nicotiana plumbaginifolia*, which is deficient in phytochrome chromophore biosynthesis, showed increased levels of abscisic acid (ABA) (Kraepiel et al., 1994), one of the most important hormones involved in this kind of stress. However, because the chromophore is common to all types of phytochromes, this response appears to be quite variable and species dependent. For example, Ferreira Júnior et al. (2018) observed that in the tomato mutant, also deficient in chromophore biosynthesis (*aurea* or *au* mutant), it was more sensitive to water deficit.

The specificity of phytochromes has become increasingly evident, especially to the type B family of these photoreceptors. For example, compared to the control, Arabidopsis *phyB* mutants, which are deficient in phytochrome B biosynthesis, showed a reduction in stomatal conductance under water deficit conditions (Boggs et al., 2010). Furthermore, in the same species, Allen et al., (2019) observed an increase in dehydration tolerance in mutants with deficiency in phytochrome B due to the lower relative water loss in detached leaves. Interestingly, this evidence also reaches plants with agronomic interest, since, in rice (*Oriza sativa* L. cv Nipponbare), plants deficient in phyB showed greater tolerance to water deficit compared to the control genotype, and despite having shown a reduction in net carbon dioxide assimilation due to reduced leaf area, stomatal density and transpiration, the decrease in photosynthetic rate did not affect grain production (Liu et al., 2012). In tomato, Gavassi et al. (2017) observed that 10-day-old seedlings of the *phyB1* mutant, submitted to low osmotic potential, presented an increase in the concentration of chlorophylls and carotenoids, as well as the greater length of hypocotyl and root in relation to control genotype. Interestingly, part of these responses was followed by a greater accumulation of proline and glycine-betaine in the *phyB1* mutant.

According to the above evidence, although the molecular mechanisms depending on the phytochrome B family are poorly understood, so far this type of phytochrome seems play an important role in control responses to water deficit. Thus, it is reasonable to conjecture that the phyB type of phytochromes can modulate the

responses to water deficit during different stage growth, a fact that is highly important for agronomic species such as tomato, but which is not yet known. In this species, to date, the knowledge about the effects of the *phyB1* mutant shows that, under well hydrated conditions, there was an increase in production of 74% and 39% of fruits both in the greenhouse and in the field, respectively, with no decrease in physicochemical quality compared to the control genotype (Alba et al., 1999). In addition, previous studies demonstrated that phytochromes are part of responses to water deficit in tomato (D'Amico-Damião et al., 2015), with phyB1 being one of the main modulators (Gavassi et al., 2017). However, so far it is not known whether these favorable responses identified in tomato seedlings deficient in phyB1 will have any effect on fruit production or even on their physicochemical characteristics. Thus, considering the economic importance of tomatoes, this unprecedented proposal will make it possible to elucidate the participation of phyB1 in tomato production under water deficit conditions, the stressor that has been a matter of concern due to evident climate changes. Moreover, considering the plant as a complex system that perceives environmental changes, especially water deficit, we hope not only to confirm or reject the presented hypothesis, but to raise new questions that can be explored by the scientific community, and create a new approach for the plant improvement. The objective of this work is to verify whether phytochrome B1 of tomato is part of the responses to water deficit in fruit production.

2. MATERIAL AND METHODS

2.1. Plant material and growth conditions

The use of mutants in phytochromes is a great tool to understand the functions of these photoreceptors throughout vegetative and reproductive development, given the unique effect of genetic components (alteration in phytochrome biosynthesis). Therefore, for this proposal, was used the tomato (*Solanum lycopersicum* L.) mutant deficient in phyB1 biosynthesis (*temporary red light insensitive – tri*) which presents as phenotypical characteristic etiolated seedings under red light (van Tuinen et al., 1995) and its isogenic line wild-type (WT) of cv Moneymaker. Seeds were kindly provided by the "Tomato Genetics Resource Center" (TGCR; Davis – California). To avoid any

contamination, seeds were pre-treated with a 5% sodium hypochlorite solution for 10 min and subsequently well washed before sown. The sowing was realized in trays containing a 1:1 (v/v) mixture of a commercial substrate (BioPlant, Brazil) and expanded vermiculite, and then, left to grow in a chamber with artificial illumination and 12h photoperiod. Fifteen days after sowing (15 DAS), seedlings were transferred to 12 L pots filled with 12 kg of typical dystrophic oxisol supplemented with 4.8 g P_2O_5, 0.018 g B, 0.36 g N, and 1.8 g K_2O. The maintenance fertilizations were made once a week, for 16 weeks applying 1/16 part of the total requirement of N (300 kg ha^{-1}) and K_2O (240 kg ha^{-1}), totalizing 0.1125 g pot^{-1} N and 0.09 g pot^{-1} K_2O in each fertilization, following the recommendation of Raij et al. (1996) for staked-tomato fertilization. Foliar fertilization of Ca was performed weekly applying 6 g L^{-1} $CaCl_2$, as well as pest and disease control with abamectin (0.7 mL L^{-1} of Abamectin Nortox), acetamiprid (0.4 g L^{-1} of Mospilan WG), and metiram + pyraclostrobin (4 g L^{-1} Cabrio® Top). The plants were conducted in simple stem with the removal of all lateral buds and maintained in a greenhouse at São Paulo State University – Jaboticabal/SP, Brazil (21°14' 25.35" S, 48° 17' 10.90" O) with an average of the maximum and minimum temperature, respectively, 38.36°C (SE ± 0,57°C) and 18.88°C (SE ± 0.40°C), and maximum and minimum relative humidity, respectively, 87.63% (SE ± 1.11%) and 23.10% (SE ± 1.19%). All plants were watered daily throughout the experimental period (135 days after sowing – DAS) accordingly its irrigation treatment. Two experiments were carried out simultaneously, with the only difference between them the growth stage in which the water deficit stress began (Figure 1 of appendix A).

2.2. Experimental design and irrigation treatment

Two experiments were carried out in entirely randomized factorial with 4 replicates in which factor 1: genotype *phyB1* mutant and WT, factor 2: replacement of 100% and 50% of daily water evapotranspiration (ETP). The pots were weighed daily, and the evapotranspiration rate was determined through the difference in pot weight from the last day, and then, the irrigation was performed accordingly for each treatment (Figure 2 of appendix A). In the experiment I, the water deficit treatment was realized in the vegetative growth stage, starting 26 DAS and maintained until first symptoms of wilt. After the first symptoms of wilt, at 36 DAS, 4 plants were harvested for vegetative

growth analysis, and 4 plants left were recovered from wilt and maintained well-watered in field capacity until the end of the experimental period (135 DAS) to the analysis of fruit growth parameters and vegetative growth (Figure 1A of appendix A). The experiment II followed the same conditions previously described for experiment I, but with the water deficit treatment realized in the reproductive stage, starting at 33 DAS (first visible flower bud in 50% +1 of plants) and maintained until first symptoms of wilt, at 41 DAS (Figure 1B of appendix A).

2.3. Determination of vegetative parameters

The growth analysis of vegetative parameters in both experiments were performed at the end of water deficit treatment (I – 36 DAS; II – 41 DAS) and at the end of the experimental period (135 DAS). Stem length (m) was measured with a ruler considering from the base of the plant until apical meristem, stem diameter (m) with digital caliper between third and fourth internodes, and root length directly with ruler. Leaf area (cm^2) was evaluated using device LICOR® 104, model LI 3100. Subsequently, stem, leaves and root were oven dried at 65°C for 72h, and biomass accumulation of leaf, stem and root was quantified using analytics scale. Shoot dry weight was determined by the sum of the dry weight of the leaf and stem. The total dry weight was obtained through the sum of leaf, stem, and root, and shoot: root ratio through the division of shoot dry weight for root dry weight.

2.4. Determination of reproductive parameters

The plants were observed daily for determining numbers of days to the appearance of the first visible flower bud, days to anthesis of the first flower bud, days to first visible fruit, and days to the first fruit reach color break accordingly to color sorting methods of United States Standards for Grade of Fresh Tomatoes (USDA, 1991) in which classified color break as color from green to tannish-yellow, pink or red on not more than 10 percent of the surface.

2.5. Fruit production, quality, and morphometric analysis

Due to the genotypes used in this research presented indeterminate growth habit, fruits were harvested as soon as they ripen, considered ripe fruits when reached the turning-color classified by United States Standards for Grades of Fresh Tomatoes (USDA, 1991) in which more than 10% but not more than 30% of the surface, in the aggregate, shows a definite change in color from green to tannish-yellow, pink, red, or a combination thereof. Longitudinal and transverse diameter (m) of ripe fruits were measured using digital caliper. All harvested ripe fruit through the experimental period (135 DAS) were counted and weighted to quantify the number and fresh weight of ripe fruit (g), and then, were oven dried at 65° C until reaching constant weight to evaluating the dry weight of ripe fruit (g) using the analytic scale. At 135 DAS all unripe fruits were harvested, and the same procedure was made to determine the number, fresh and dry weight of unripe fruit. The total number, total fresh and dry weight of fruit were estimated by the sum of values from ripe and unripe fruit production. Total soluble solid (°Brix) was determined with digital refractometer ATAGO® PR-101α. The last analyse was made only in fruits that reached light-red or red color in more than 90 percent of the surface.

2.6. Data analyses

The assumptions of the analysis were tested, that is, the normality of the errors (Shapiro-Wilk) and homoscedasticity (Levene), subsequently data were subjected to analysis of variance ANOVA (significance at $p<0.05$). Means values were compared using Tukey's test (significance at $p<0.05$) in AgroEstat software (www.agroestat.com). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment. In figures, the graphics were plotted using original data accompanied by standard errors (SE).

3. RESULTS

3.1. Water deficit stress in vegetative growth stage

3.1.1. Vegetative growth analysis

At the end of the water deficit treatment (36 DAS), the 50% ETP treatment impaired plant growth in comparison with control treatment (100% ETP) in the WT genotype for the characteristic of the dry weight of leaf (Figure 1A), stem (Figure 1C), shoot (Figure 1D) total (Figure 1B). While *phyB1* mutant only presented difference in stem dry weight that was higher in 50% ETP compared to 100% ETP. Interestingly, WT showed higher root dry weight in 50% ETP compared to 100% ETP, such a pattern was not verified in *phyB1* mutant that no differences was observed in such conditions (Figure 1E). Proportionally, WT genotype had a higher investment in roots development under water deficit conditions as verified by the lower shoot: root ratio that was lower in 50% ETP compared to 100% ETP (Figure 1F). Despite *phyB1* mutant did not change leaf, root, and shoot growth between 100% ETP and 50% ETP treatments, the higher shoot: root ratio (Figure 1F) clearly indicated that in the water deficit condition this mutant did not spend as many resources for root development as WT.

Dry weight of leaf (Figure 1A), root (Figure 1E) and total dry weight (Figure 1B) was equal between *phyB1* mutant and WT in 100% ETP treatment. However, in the 50% ETP treatment the *phyB1* mutant presented higher leaf (Figure 1A), stem (Figure 1C), shoot (Figure 1D) and total dry weight (Figure 1B) in contrast with WT. Notably, the *phyB1* mutant has as genotypical characteristic a higher stem accumulation, since in all treatments this mutant showed increased stem dry weight compared to WT (Figure 1C). However, in water deficit conditions, *phyB1* mutant seems allocated even more resources on stem development considering the increased stem dry weight in the 50% ETP treatment compared to 100% ETP treatment (Figure 1C).

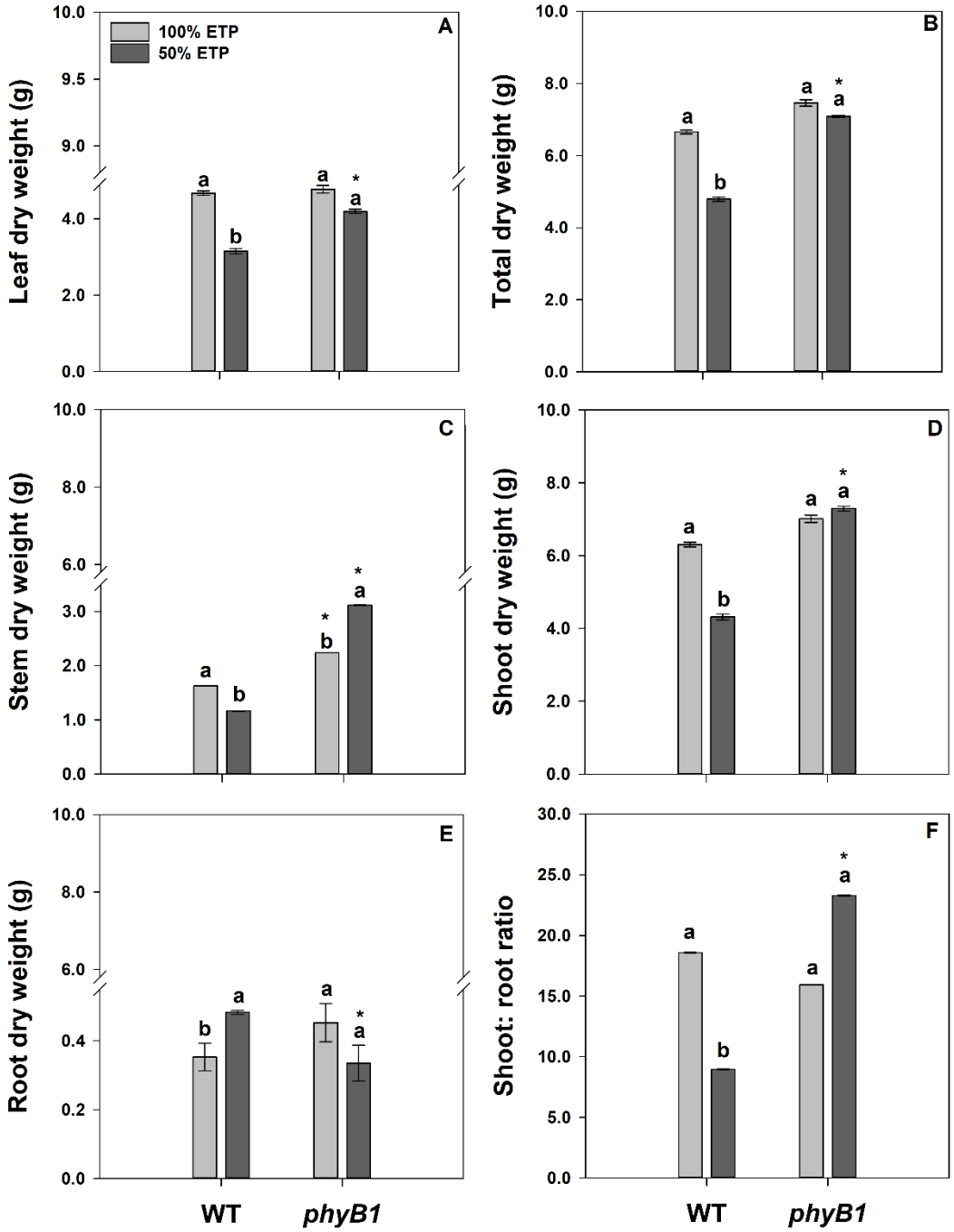

Figure 1. Vegetative growth analysis of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 10 days in vegetative growth stage, starting at 26 days after sowing (DAS) and ending at 36 DAS. Leaf dry weight (A), total dry weight (B) stem dry weight (C), shoot dry weight (D), root dry weight (E) and shoot: root ratio (F). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

As showed before, in water deficit condition *phyB1* mutant increased their leaf dry weight compared to WT, such improvement was also verified in terms of leaf area, since differences was seen between leaf area of *phyB1* and WT in 50% ETP treatment (Figure 2A). The increased stem growth in *phyB1* mutant was followed by stem stretching instead of stem thickening, as shown by the higher stem length of *phyB1* mutant (Figure 2B) and equal stem diameter (Figure 2C) in all treatments compared to WT (Figure 2B). Additionally, the differences seen in root dry weight were not displayed in root length that showed the same values for both genotypes in all treatments (Figure 2D).

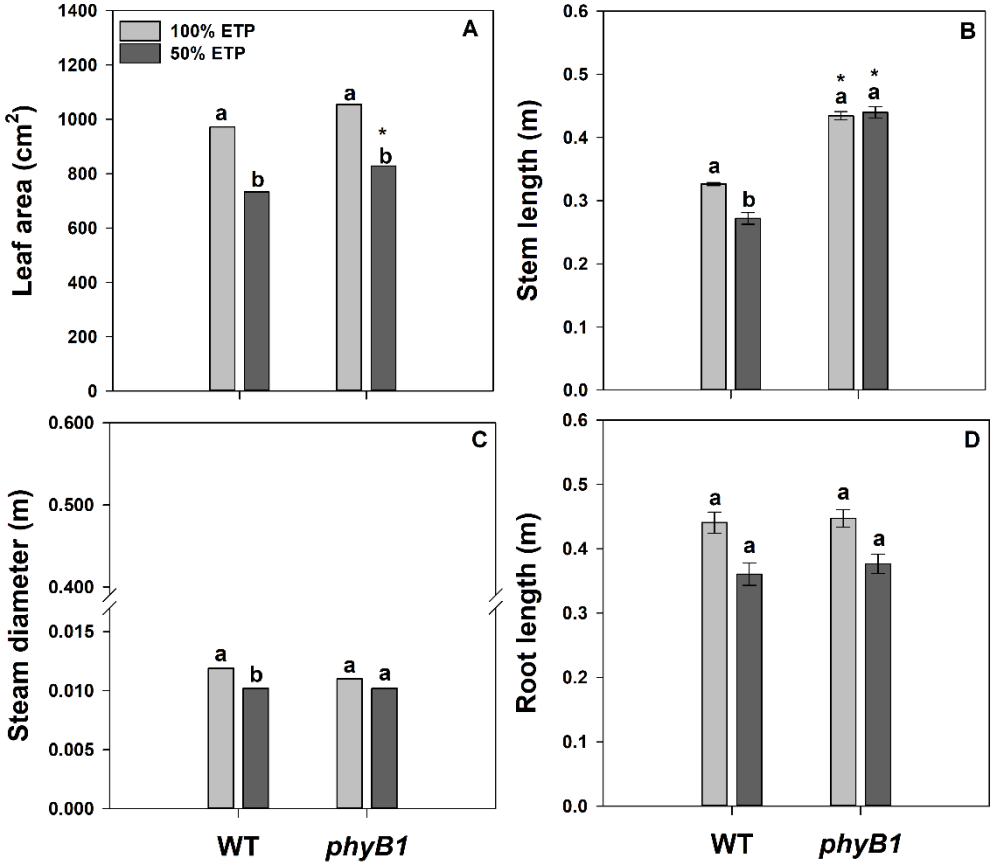

Figure 2. Vegetative growth analysis of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 10 days in vegetative growth stage, starting at 26 days after sowing (DAS) and ending at 36 DAS. Leaf area (A), steam length (B), steam diameter (C) and root length (D). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

3.1.2. Reproductive parameters

On absolute average, the *phyB1* mutant in comparison to WT, presented a delay to the appearance of the first fruit (2.75 days) and for the first fruit to reach color break (2.13 days). The water deficit treatment presented effect only in the appearance of the first fruit in the color break, in which *phyB1* presented a longer cycle in comparison to WT in 100% ETP while in 50% ETP no differences were observed (Table 1).

Table 1. Reproductive parameters of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) at vegetative stage growth. The mean (n=4) values were compared by Tukey's test (p≤0.05). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

Parameters	Genotypes				Absolute mean	
	WT		*phyB1*		WT	*phyB1*
	100%	50%	100%	50%		
1st Flower bud	14.25 a	14.50 a	14.75 a	14.66 a	14.70	14.37^{ns}
Anthesis	24.75 a	25.00 a	26.25 a	26.00 a	24.87	26.12^{ns}
1st Fruit	29.75 a	29.25 a	33.00 a	31.50 a	29.50	32.25*
1st Fruit "break"	64.25 a	65.00 a	67.25 a*	66.25 a	64.62	66.75*

3.1.3. Fruit production, quality, and morphometric analysis

Differently to vegetative growth analysis at 36 DAS that clearly showed a better performance of *phyB1* mutant to biomass accumulation in water deficit conditions, in terms of fruit production, such superiority was not verified. The *phyB1* mutant and WT genotype presented no differences of total number of fruits (Figure 3A), total fresh weight of fruits (Figure 3B) and total soluble solid (°Brix) (Figure 3D) in all ETP treatments. However, despite the water deficit treatment did not cause a change in dry weight of fruits in either WT and *phyB1* between 100% and 50% ETP, comparison between the genotypes at 50% ETP showed that the *phyB1* displayed a lower accumulation of the dry weight of fruits (Figure 3C).

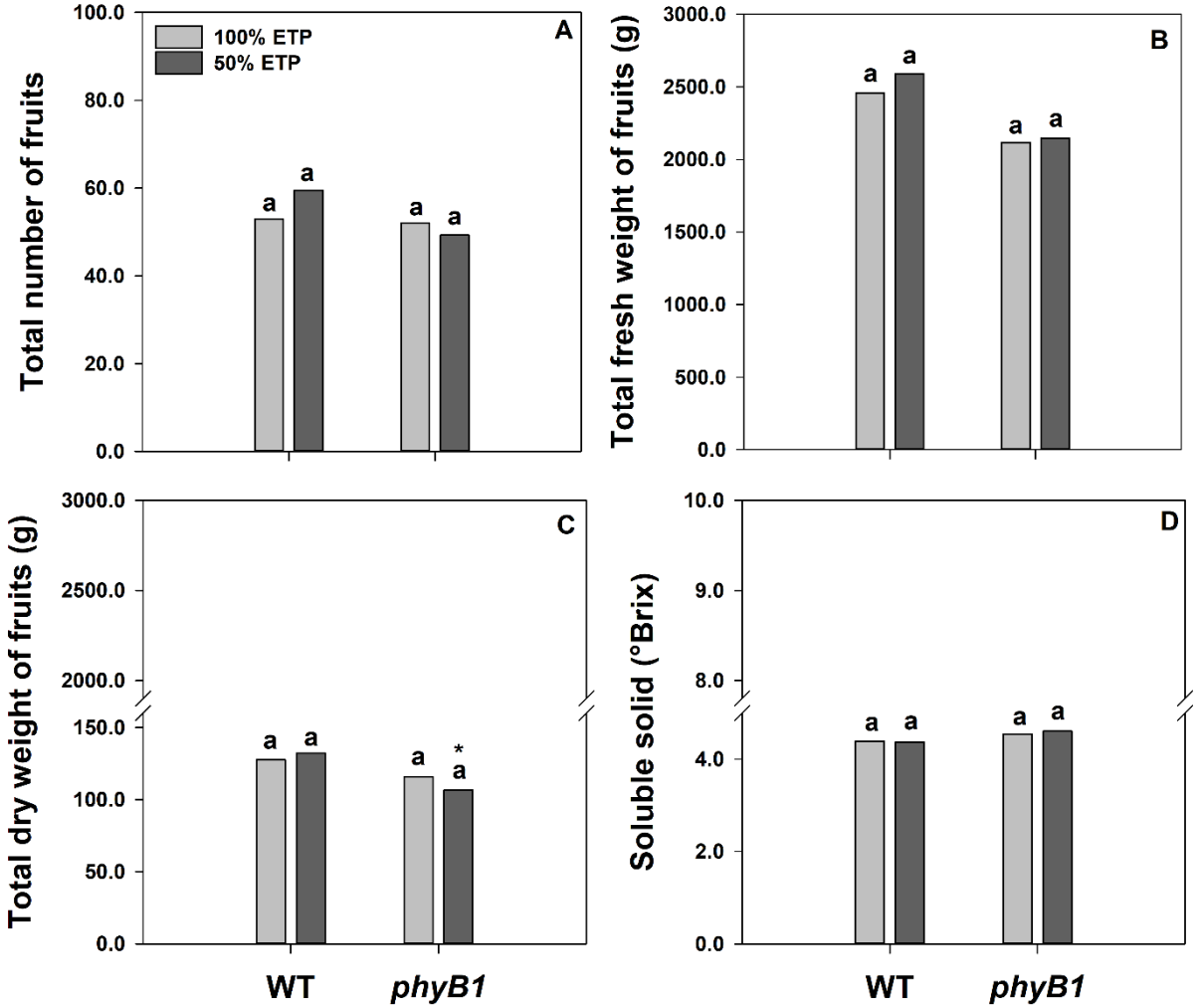

Figure 3. Fruit production and quality of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 10 days in vegetative growth stage, starting at 26 days after sowing (DAS) and ending at 36 DAS. Total number of fruits (A), total fresh weight of fruits (B), total dry weight of fruits (C) and soluble solid (°Brix) (D). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

The production of ripe fruits was lower in *phyB1* mutant in comparison to WT in all ETP treatments (Figure 4A), which is certified as genotypical characteristic of *phyB1* mutant a higher plant biomass accumulation in detriment of fruit production. Interestingly, *phyB1* presented lower average fresh weight of fruits in 100% ETP treatment compared to WT, while no difference was observed between genotypes in

50% ETP (Figure 4B). Both fruit transverse and longitudinal diameter were not affected by ETP treatments within same genotype, however, *phyB1* presented lower fruit transverse diameter in comparison to WT in 100% ETP treatment (Figure 4C) and higher fruit longitudinal diameter in comparison to WT in 50% ETP (Figure 4D).

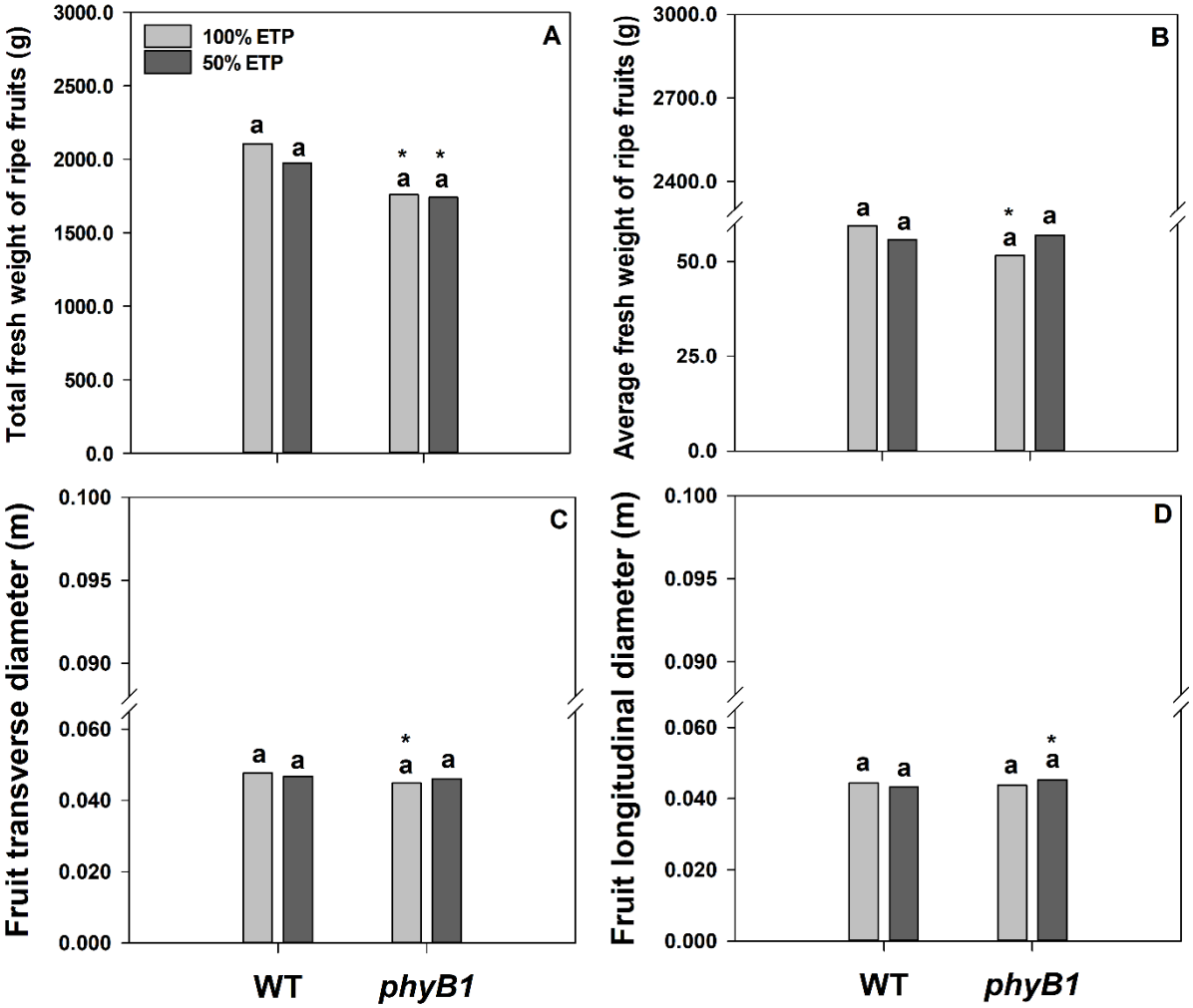

Figure 4. Fruit production and morphometric analysis of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 10 days in vegetative growth stage, starting at 26 days after sowing (DAS) and ending at 36 DAS. Total fresh weight of ripe fruit (A), average fresh weight of ripe fruits (B), fruit transverse diameter (C) and fruit longitudinal diameter (D). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

3.2. Water deficit stress in reproductive growth stage

3.2.1. Vegetative growth analysis

With de purpose to understand the influence phyB1 in tomato plants development and fruit production under water deficit conditions imposed in different growth stages. The vegetative growth analysis was performed at the end of ETP treatments (41 DAS). Within *phyB1* mutant and WT genotype, the 50% ETP treatment decreased dry weight of leaf (Figure 5A), shoot (Figure 5D) and total (Figure 5B) in comparison to 100% ETP treatment.

Nevertheless, no differences were observed for these latter features between *phyB1* and WT within each ETP treatments. Interestingly, stem dry weight accumulation in *phyB1* mutant showed no difference between 100% ETP and 50% ETP treatment (Figure 5C), unlike when 50% ETP treatment happened in the vegetative growth stage that showed an increase in stem dry weight in 50% ETP treatment (Figure 1C). In addition, is worth noting that in both, 100% ETP treatment and 50% ETP treatment, stem dry weight was equal between *phyB1* mutant and WT. Root dry weight was not altered between and within both genotypes in any ETP treatments (Figure 5E), which leads to suggest that the reduction in shoot: root ratio (Figure 5F) presented by *phyB1* mutant in 50% ETP treatment compared to 100% ETP treatment is due to reduction in shoot biomass accumulation (Figure 5D).

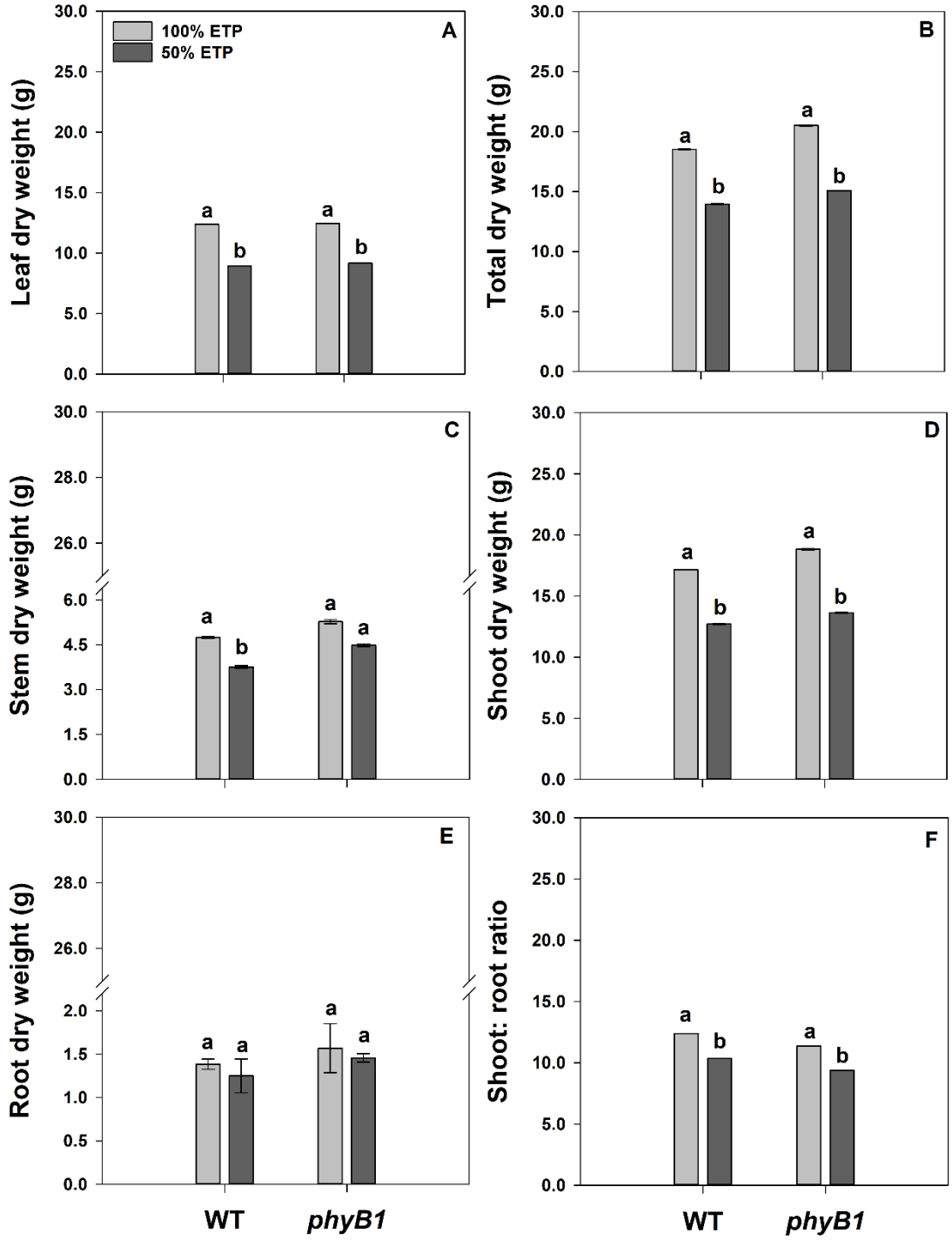

Figure 5. Vegetative growth analysis of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 8 days in reproductive growth stage, starting at 33 days after sowing (DAS) and ending at 41 DAS. Leaf dry weight (A), total dry weight (B), stem dry weight (C), shoot dry weight (D), root dry weight (E) and shoot: root ratio (F). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

Although in the water deficit condition the dry weight of leaf was equal between *phyB1* mutant and WT in 50% ETP treatment, the leaf area was higher in the *phyB1* mutant in comparison to WT in 50% ETP treatment (Figure 6A). Similarly, stem length presented higher values to *phyB1* mutant in contrast to WT in both ETP treatments. The substantial stem elongation (Figure 6B) was not due to the increase in stem biomass (Figure 5B), but possibly due to the elongation of the cells, since was not observed difference in stem dry weight, while stem diameter was reduced and equal in *phyB1* mutant in comparison to WT in 100% and 50% ETP treatments, respectively. The cell elongation pattern was also observed in roots, but in the opposite manner, since root dry weight was not altered (Figure 5C) while root length was lower in *phyB1* mutant compared to WT in 50% ETP treatment (Figure 6D), that is, water deficit stress reduces cell elongation of roots only in *phyB1* mutant.

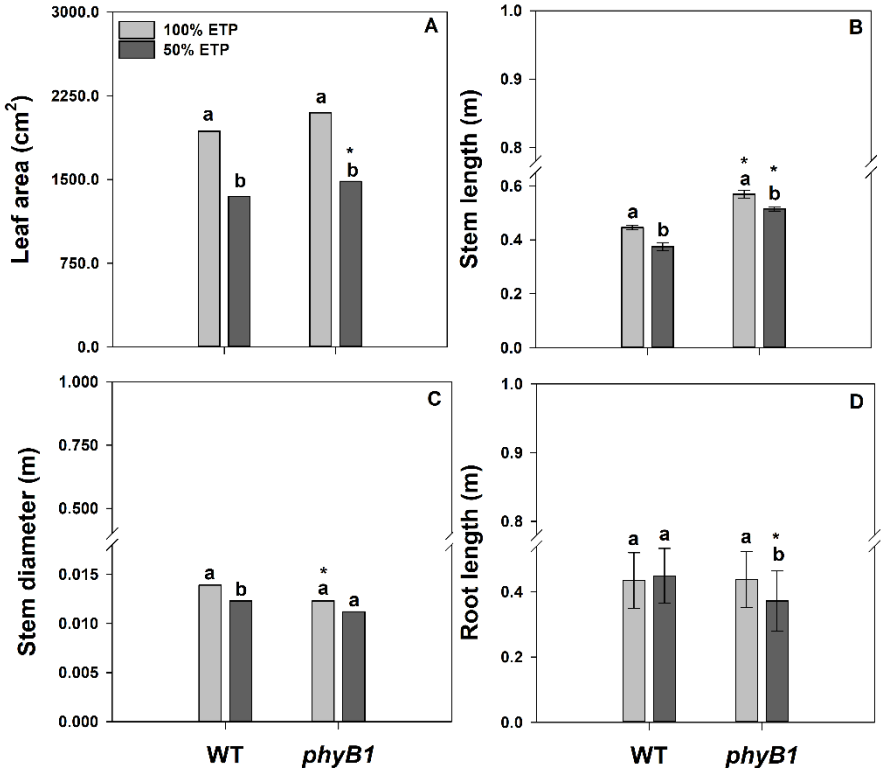

Figure 6. Vegetative growth analysis of WT and *phyB1* mutant under replacement of 100%, 75%, and 50% daily water evapotranspiration (ETP) during 8 days in reproductive growth stage, starting at 33 days after sowing (DAS) and ending at 41 DAS. Leaf area (A), steam length (B), steam diameter (C) and root length (D). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

3.2.2. Reproductive parameters

On absolute average, *phyB1* mutant and WT had no difference in the number of days to appearance of first flower bud, anthesis of first flower bud, days to first visible fruit and color breaker of first fruit (Table 2). However, was observed that *phyB1* mutant delayed the anthesis of the first flower bud (1.5 days) in comparison to WT in 100% ETP treatment. On the other hand, days to anthesis were equal between the genotypes in 50% ETP treatment. Thus, the *phyB1* presented an escape strategy, that is, when faced drought stress condition in reproductive stage growth, the mutants accelerated flower anthesis to avoid a potential reduction of descendants' generation.

Table 2. Reproductive parameters of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) at reproductive stage growth. The mean (n=4) values were compared by Tukey's test (p≤0.05). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment

Parameters	Genotypes				Absolute mean	
	WT		*phyB1*		WT	*phyB1*
	100%	50%	100%	50%		
1st Flower bud	14.25 a	14.75 a	16.25 a	14.75 a	14.50	15.50^{ns}
Anthesis	25.25 a	26.00 a	26.75 a*	25.75 a	25.62	26.25^{ns}
1st Fruit	31.75 a	31.25 a	32.25 a	32.75 a	31.50	32.50^{ns}
1st Fruit "break"	67.75 a	67.50 a	69.00 a	69.25 a	67.62	69.12^{ns}

3.2.3. Fruit production, quality, and morphometric analysis

The source-sink relationship between vegetative growth and fruit production is different according to the stage growth in which water deficit stress happens. As already showed, *phyB1* mutant presented higher vegetative growth in detriment to fruit production when water deficit treatment was performed in vegetative stage growth. But curiously, when the water deficit treatment happened in reproductive stage growth, no differences were observed in vegetative biomass accumulation between *phyB1* mutant and WT within all ETP treatments (Figure 5B), whereas *phyB1* mutant exhibited lower production of total fresh and total dry weight fruit in comparison to WT in 50% ETP

treatment (Figure 7B, Figure 7C). The accumulation of the total dry weight of fruits seems to be genotype-dependent since *phyB1* mutant presented the same values within all ETP treatments but was inferior when compared to WT in 100% and 50% ETP treatment (Figure 7C).

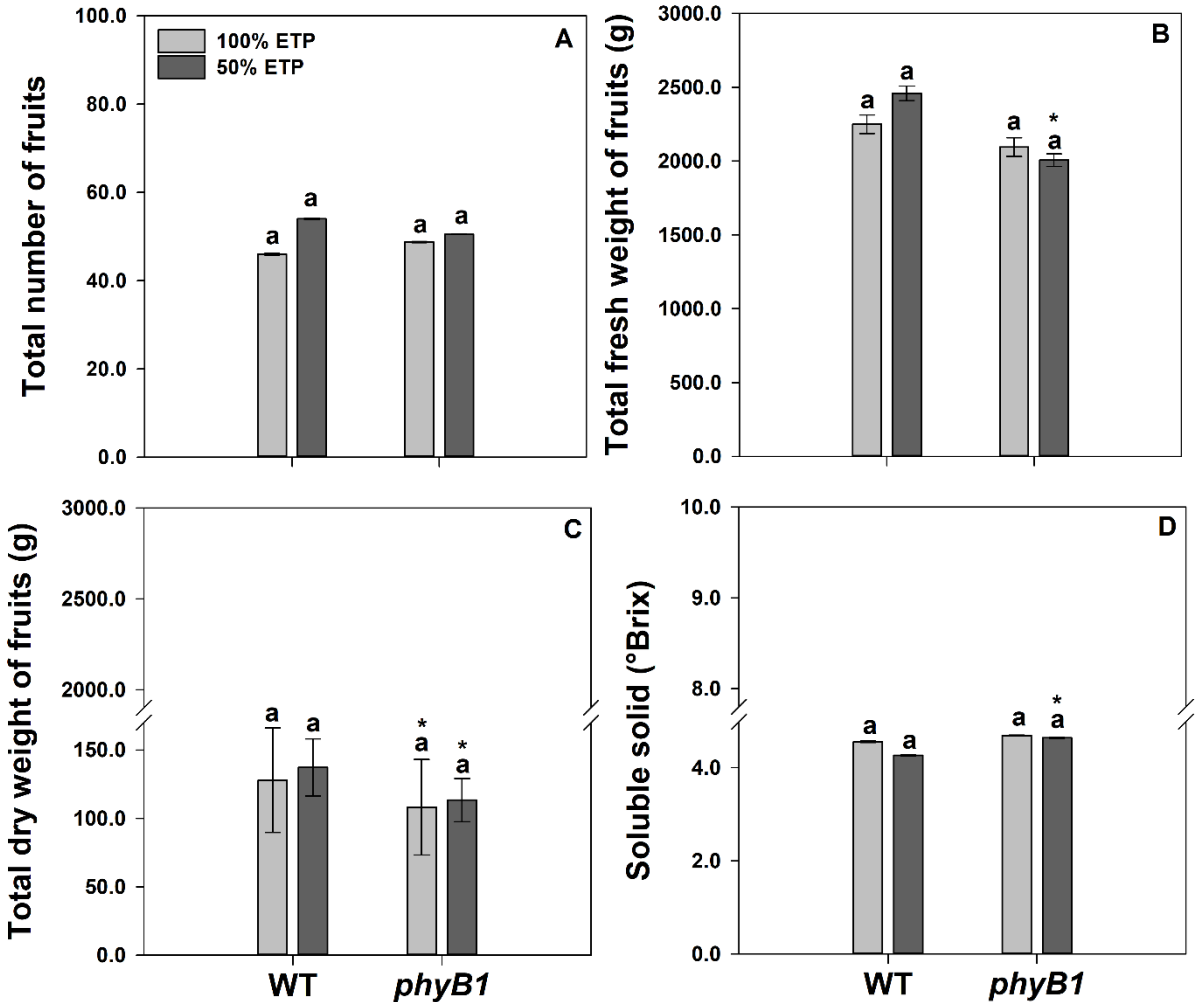

Figure 7. Fruit production and quality of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 8 days in reproductive growth stage, starting at 33 days after sowing (DAS) and ending at 41 DAS. Total number of fruits (A), total fresh weight of fruits (B), total dry weight of fruits (C) and soluble solid (°Brix) (D). The mean values were compared by Tukey's test ($p \leq 0.05$). The bars represent ± SEs (n=4). Distinct letters indicate differences between ETP treatments within each genotype and asterisk (*) mean differences between genotypes within each ETP treatment.

Despite the lower performance presented by *phyB1* mutant in water deficit conditions to the production of fresh weight of ripe fruit (Figure 8A), the total soluble solid (°Brix) was enhanced in comparison to WT in 50% ETP treatment (Figure 7D). Possibly such result in °Brix value was due to the lower size presented by the fruits of *phyB1* mutant in 50% ETP treatment as verified by the lower average fresh weight of ripe fruit (Figure 8B), and fruit transverse diameter (Figure 8C) in comparison to WT.

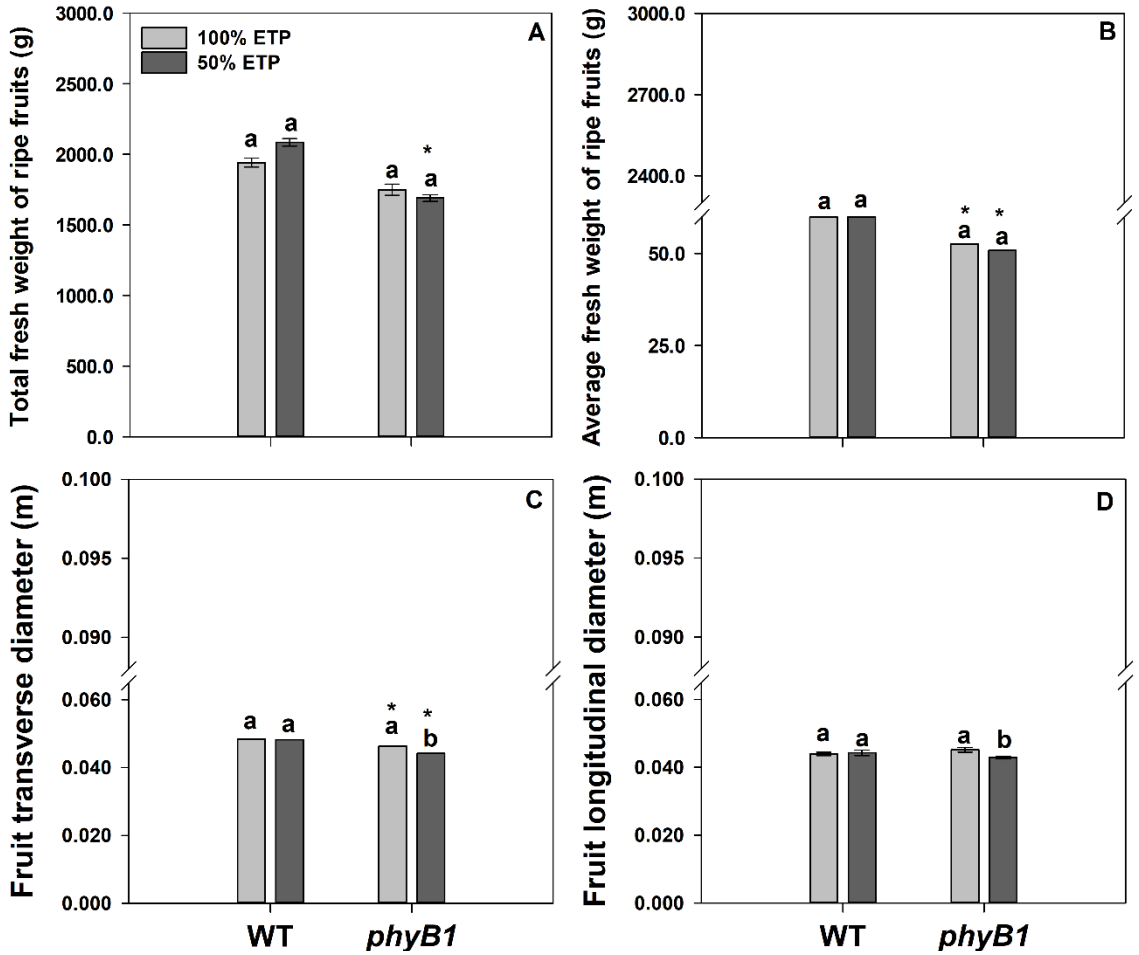

Figure 8. Fruit production and morphometric analysis of WT and *phyB1* mutant under replacement of 100% and 50% daily water evapotranspiration (ETP) during 8 days in reproductive growth stage, starting at 33 days after sowing (DAS) and ending at 41 DAS. Total fresh weight of ripe fruits (A), average fresh weight of ripe fruits (B), fruit transverse diameter (C) and fruit longitudinal diameter (D). The bars represent ± SEs. The distinct letters indicate significant differences between genotype at the same ETP, and within ETP at the same genotype. The mean values were compared by Tukey's test ($p \leq 0.05$).

4. DISCUSSION

The phytochrome B type-coordinated plant development has been widely described in the literature, especially in the model plant Arabidopsis (Franklin and Quail, 2010; Whitelam and Devlin, 1997). Despite its short cycle and the ease to manipulation, the most results obtained from Arabidopsis studies is limited to characterization of seedlings or young plants, since there is no production of commercial fruits in this specie. Fortunately, studies about phyB-mediated plant development and yield also broad tomato plants (Casal, 2013; Gupta et al., 2014 Kendrick et al., 1997), one of the most important vegetable to human food security and nutrition (Canene-Adams et al., 2005; Dorais et al., 2008), turning it an excellent model to study physiological events since seed germination to fruit production. In addition to phyB importance to photomorphogenesis, evidence have associated phyB to responses triggered by drought stress in tomato (D'Amico-Damião et al., 2015; Gavassi et al., 2017), however further studies are required to elucidate whether such phyB responses to drought condition prevail until the end of plant cycle, influencing fruit yield and quality. Therefore, tomato *phyB1* mutant was used in this study to provide knowledge to fulfill this information gap and for better understanding the phyB1 responses in vegetative growth as well as its source-sink relationship between plant biomass and harvestable organs. Thus, tomato phyB1-deficient mutant and its near isogenic line (WT) were cultivated under different water deficit regimes in vegetative growth stage (start: 26 DAS, end: 36 DAS) and reproductive growth stage (start: 33 DAS, end: 41 DAS), but being maintained well hydrated until fruit production.

4.1. Responses to drought in early developmental stages are mediated by phyB1

phyB1 mutant and WT were submitted to treatments of water deficit through different replacement of daily water evapotranspiration (100% and 50% ETP) in vegetative stage growth, beginning in 26 DAS and ending 36 DAS. Was expected reduction in tomato biomass accumulation under water deficit condition, as shown by Morales et al. (2015) that verified a decrease in dry weight of leaf, stem, and root of tomato plants. Such pattern was observed in WT that lowered total dry weight between

100% ETP and 50% ETP treatments (Figure 1B). Differently, *phyB1* mutant presented no changes in total dry weight within ETP treatments, however, when compared to WT, its value was higher in 50% ETP treatment (Figure 1B). These results suggest that phyB1 act as negative regulator to drought responses, at least in terms of shoot growth, since leaf (Figure 1A), and shoot dry weight (Figure 1D) were also higher in *phyB1* mutant contrasted to WT in 50% ETP, while no differences were observed in 100% ETP between WT and *phyB1*. In fact, WT plants exhibited a reduced leaf area (Figure 2A) and stem dry weight (Figure 1C) that was accompanied by the reduction in stem length in 50% ETP compared to 100% ETP (Figure 2B). On the other hand, despite water deficit treatment increased stem dry weight in *phyB1* mutant in 50% ETP compared to 100% ETP, such differences were not observed in terms of stem length (Figure 2B) and stem diameter (Figure 2C) were similar between 100% and 50% ETP treatments. The enhanced stem development in *phyB1* mutant is a genotypical feature, once the lack of phyB1 molecules makes plants show a stem overgrowth due to the syndrome called “shade-avoidance” (Franklin and Whitelam, 2005).

Facing water deficit conditions, plants present different mechanisms to avoid excessive water loss through adjustments mainly in water absorption, transport, or loss by transpiration. In terms of water transport, a report revealed that phyB in cucumber (*Cucumis sativus*) controls positively xylem conductivity since *phyB* mutant presented lower diameter and number of xylem vessels (Casal et al., 1994). Despite to date is not known whether such response would be conserved to phyB1 in tomato, studies demonstrated that *phyA* mutant in tomato presented lower stem-specific hydraulic conductivity, being associated to a lower number and transversal area of xylem vessels number (Auge et al., 2012). Thus, although further analysis is required to understand phyB1 signalling in tomato xylem development, would be well accepted to suggest that phyB1 possibly regulates positively vessel conductivity in tomato plants and influences water transportation.

In addition, phyB1 signalling also reaches water absorption, since our study showed that phyB1 acts was positive regulator of root growth during drought stress in vegetative growth stage of tomato. The WT presented an increase in root dry weight at 50% ETP treatment compared to 100% ETP treatment, while in *phyB1* root dry

weight showed no difference within ETP treatments but was lower than WT in 50% ETP treatment (Figure 1E). Thus, the only adjustment that seems to be beneficially regulated in *phyB1*, in order to confer tolerance to water shortage, is water loss by transpiration.

Many studies have revealed that phyB molecule act as a positive regulator of stomatal conductance in Arabidopsis (Boggs et al., 2010; González et al., 2012) and phyB1 in tomato (D'Amico-Damião et al., 2015). Moreover, evidence showed that seedling of tomato *phyB1* mutant submitted to low water potential increased the proportion of osmoprotectants (e. g. proline and glycine-betaine) (Gavassi et al., 2017). Thus, considering the impact of phyB1 lacking in tomato, could be suggested that *phyB1* mutant at the same time had a lower conductivity of xylem vessels and root development, that is, presented impaired water uptake and transport. On the other hand, attempting to alleviate such negative points, *phyB1* was described to reduce water loss by the reduction of stomatal conductance. The possible reduction in water loss in leaves was not enough to mitigate the impaired water uptake and transport, which explains our finds that showed *phyB1* mutant wilting before WT at 50% ETP (Figure 9).

To summarize, plants possess many mechanisms to avoid or escape drought conditions, here, despite *phyB1* mutant wither before WT (Figure 9) and have spent resources on shoot development at expenses of the root, as seen by the higher shoot: root ratio exhibited by *phyB1* mutant compared to WT in 50% ETP treatment (Figure 1F). The greater accumulation of total biomass in *phyB1* mutants in comparison to WT at 50% ETP, while in 100% ETP no differences were observed between the genotypes (Figure 1B) highlights phytochrome B1 of tomato as a negative regulator of responses to drought stress during vegetative stage growth.

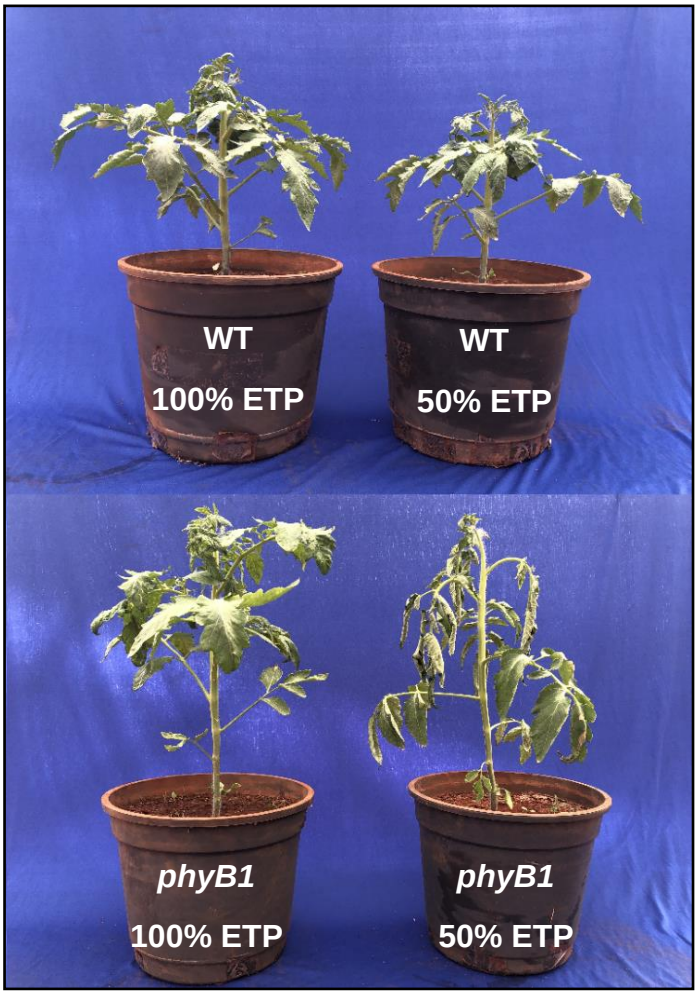

Figure 9. Tomato plants of WT and *phyB1* mutant at 36 days after sowing (DAS), under replacement of 100% and 50% daily water evapotranspiration (ETP) during 10 days in vegetative growth stage, starting at 26 DAS and ending at 36 DAS. Note at the end of the water deficit period *phyB1* mutant wilted while WT kept its turgor in the 50% ETP treatment.

Another strategy presented by plants to deal with scarcity of water resources is achieved through a shortening of the life cycle that allows plants to reproduce before the environment dries up (Farooq et al., 2009). Generally, the cycle of plants is determined interactively by genotype and the environment and determines the crop's ability to escape climatic stresses, including drought (Dingkuhn and Asch, 1999). Here, we found that drought condition did not alter the cycle of WT and *phyB1* mutant, since comparison among ETP treatments within the same genotype were equal for number

of days to appearance of first visible flower bud, anthesis, first fruit and color breaker of first fruit (Table 1). However, considering the absolute mean, *phyB1* mutant presented an increased in number of days to the appearance of first fruit and for color breaker of first fruit in comparison to WT (Table 1), setting phyB1 as positive regulator of flowering and fruit development, at least in terms of number of days.

In fact, molecular studies have revealed that flowering is positively phyB1-mediated, for example, Cao et al. (2016) showed that floral inhibitor genes *FT*-like (*FLOWERING LOCUS T*) (Karlgren et al., 2011) named *SlSP*$_5$*G*$_2$ and *SlSP*$_5$*G*$_3$ were up-expressed in tomato *phyB1* mutant. In addition, helix-loop-helix (bHLH) transcription factor inhibited by phytochrome, such as PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) was described to delay fruit ripening in tomato (Rosado et al., 2019). Nevertheless, further studies are required to understand the underlies of downstream pathways phyB1-modulated, since Gupta et al. (2014) found that tomato *phyB1* mutant accelerated fruit development and ripening.

Similarly, phyB1 did not modulate responses to drought condition for tomato fruit production and quality, given both WT and *phyB1* mutant showed no differences in total number of fruits (Figure 3A), total fresh weight of fruits (Figure 3B) and total soluble solid (°Brix) (Figure 3D) among ETP treatments within the same genotype. However, *phyB1* mutant in comparison to WT seems to possess as genotypical characteristic lower increment of total dry weight of fruit (Figure 3C), fresh weight of ripe fruit (Figure 4A) and fruit size verified by the lower average fresh weight of fruit (Figure 4B) and fruit transverse diameter (Figure 4C). Different results were described by Alba et al. (1999) that showed an increase in fruit yield of tomato *phyB1* mutant compared to WT.

The source-sink partitioning is strongly related to crop yield, since the balance between assimilate production and consumption must be very regulated whether improvement in productivity is desired (Rossi et al., 2015). Therefore, we suggest that the lower production presented by *phyB1* mutant can be assigned to its preference to invest in vegetative growth at the expenses of fruit production, must be taken to account that, possibly, the greater difficulty of this mutant in transport assimilates, due

to the impaired development of conductor vessels, constrained the fruit yield. Once photoassimilates transport is slowed, the sink strength is impaired, which leads to the accumulation of carbohydrates in the leaves and then decreases photosynthesis and, later, fruit production (Adams III et al., 2013).

4.2. Drought stress in reproductive stage changes source-sink partitioning dependent on phyB1

For a better understanding of responses to drought stress phyB1-modulated, the treatments of ETP were also performed when more than 50% of plants presented visible flower bud (starting at 33 DAS; ending at 41 DAS), which marks the beginning of reproductive stage growth. It is well known that the growth stage by which the scarcity of water occurs is crucial to determine how prejudicial the stress will be in yield reduction. Generally, in grain crops, drought stress at flowering stage results in barrenness mainly due to the reduction in assimilate flux to developing ear and sustain optimal grain growth (Yadav et al., 2004). The data revealed that in maize, drought stress is more harmful when it occurs in reproductive growth stage than in vegetative with a reduction up to, respectively, 47-70% and 25-60% (Atteya et al., 2003). Yet, water deficit stress in tomato at reproductive growth stage was also described to reduce yield (Chen et al., 2013; Cui et al., 2020).

Although data suggests a harmfulness of drought stress at reproductive stage growth, in this work, no substantial changes were found in terms of vegetative biomass accumulation and fruit yield, since most patterns presented by *phyB1* mutant and WT within ETP treatment were kept between the two moments of water deficit stress, in vegetative growth stage (Figure 1 and Figure 2) and reproductive growth stage (Figure 5 and Figure 6). However, the slight differences found can be assigned to the fact that once having flower buds in development, source-sink relationship changes to provide conditions to flowering. This fact possibly reduced the leaf dry weight of *phyB1* (Figure 5A), stem dry weight (Figure 5C), shoot dry weight (Figure 5D) and total dry weight (Figure 5B) in the 50% ETP treatment in comparison to 100% ETP. Furthermore, *phyB1* mutant presented equal stem accumulation in comparison to WT in 100%, and 50% ETP no difference was found. The presence of flower bud in development clearly

alters the strategy mechanism to drought tolerance, since the plants stopped to invest on root development as verified by the same root dry weight between and inside WT and *phyB1* mutant in all ETP treatments. Being older and bigger, plant requires more water to keep its turgor, thus, both WT and *phyB1* mutant wilted in the end of deficit treatment (41 DAS) (Figure 10), although *phyB1* mutant presented higher leaf area compared to WT in 50% ETP treatment (Figure 6A).

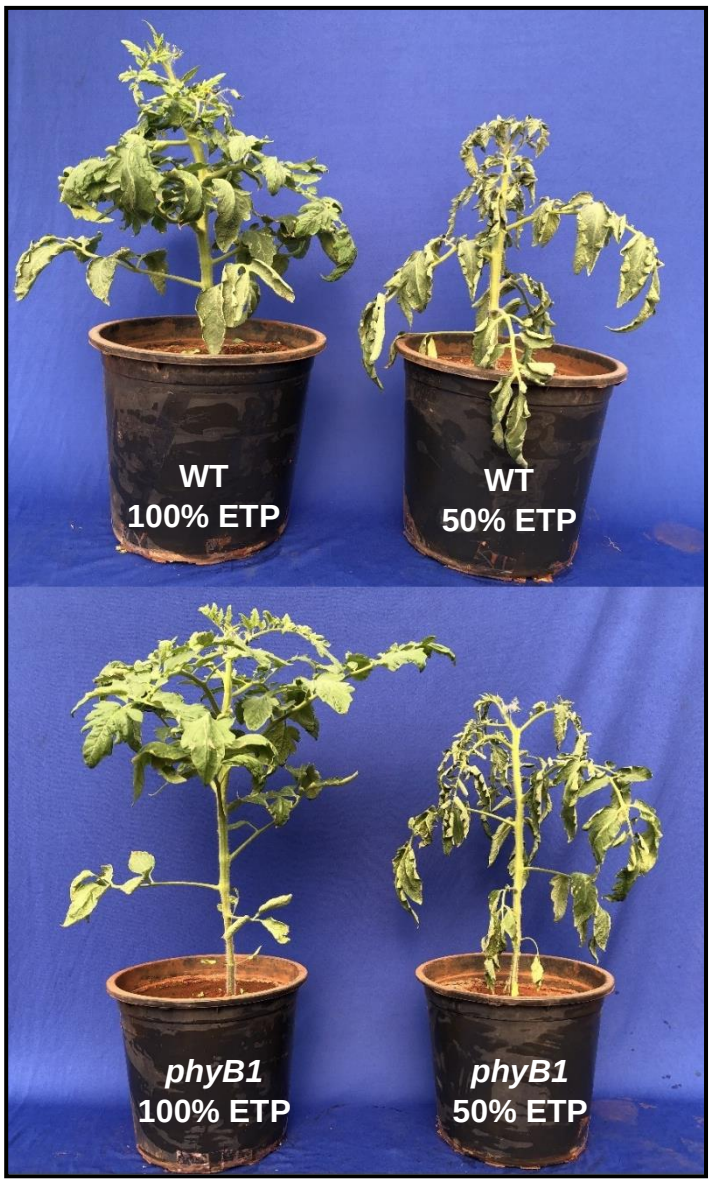

Figure 10. Tomato plants of WT and *phyB1* mutant at 41 days after sowing (DAS), under replacement of 100% and 50% daily water evapotranspiration (ETP) during 8 days in vegetative growth stage, starting at 33 DAS and ending at 41 DAS. Note that under drought stress condition both genotypes wilted.

Likewise, phyB1 did not influence tomato life cycle when water deficit treatment was carried out in reproductive stage (Table 2), however, it is worth to note that phyB1 downstream pathways still induced flowering given the delay to appearance of anthesis in the first flower bud presented by *phyB1* mutant in relation to WT in 100% ETP treatment (Table 2). In addition, the lower total fresh weight of fruit presented by *phyB1* mutants in comparison to WT in 50% ETP (Figure 7B) and total dry weight of fruit (Figure 7C) was followed by the reduction in fruit size, verified by the lower average fresh weight of fruit (Figure 8B) and fruit transverse diameter (Figure 8C) in *phyB1* mutants compared to WT in 50% ETP treatment. It is well known that fruit development begins yet in floral stage (Giménez et al., 2010; Gillaspy et al., 1993), thus, water deficit stress in this stage can impact directly fruit size and yield. Therefore, these data suggest that *phyB1* presents as genotypical characteristics lower fruit production, once in either 100% or 50% ETP, *phyB1* showed lower production of fruits and size. Such reduction in fruit size reflected directly in an increase in sugar concentration of fruits, since *phyB1* presented higher total soluble solid (°Brix) compared to WT in 50% ETP treatment. These results corroborate with studies of Chen et al. (2013) and Pulupol et al. (1996) which found an increase of °Brix content in tomato fruits submitted to water shortage.

5. CONCLUSION

The phytochrome B1 of tomato modulates responses to water deficit stress accordingly the stage growth by which the water scarcity happens. In terms of vegetative biomass accumulation was shown that although phyB1 negatively modulated responses to drought stress when water deficit is performed in the vegetative growth stage, root development as well as fruit yield were positively regulated by phyB1 with no chances in fruit quality. However, stem dry weight as well fresh weight of ripe fruit was clearly influenced by genotype. Interestingly, changes in the source-sink relationship due to the floral development make it difficult to perceive responses to drought modulated by phyB1 in the accumulation of vegetative biomass when water deficit occurs in the reproductive growth stage, however, in this condition, phyB1 positively modulates fruit size, reflecting in lower accumulation of soluble solid. Such change in source-sink partitioning due the floral development makes the stem

length, average fresh weight of ripe fruit and total dry weight of fruit features influenced by genotype. Therefore, to raise tomato plants that keep their vegetative growth in water shortage without concern about fruit yield, overexpression of phyB1 would be advantageous. In the opposite way, if the priority is fruit quality, phyB1 knockout would be beneficial. This pioneering work in addition to show how phyB1 modulates responses to drought in tomato also has stablished new avenues for future research to focus on to understand every single mechanism that underlies the responses to drought in each developmental stage, and then provide solid knowledge to plant breeders raise drought-tolerant plant through genetic engineering.

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APPENDICES

Appendix A. Supplementary material for chapter 2

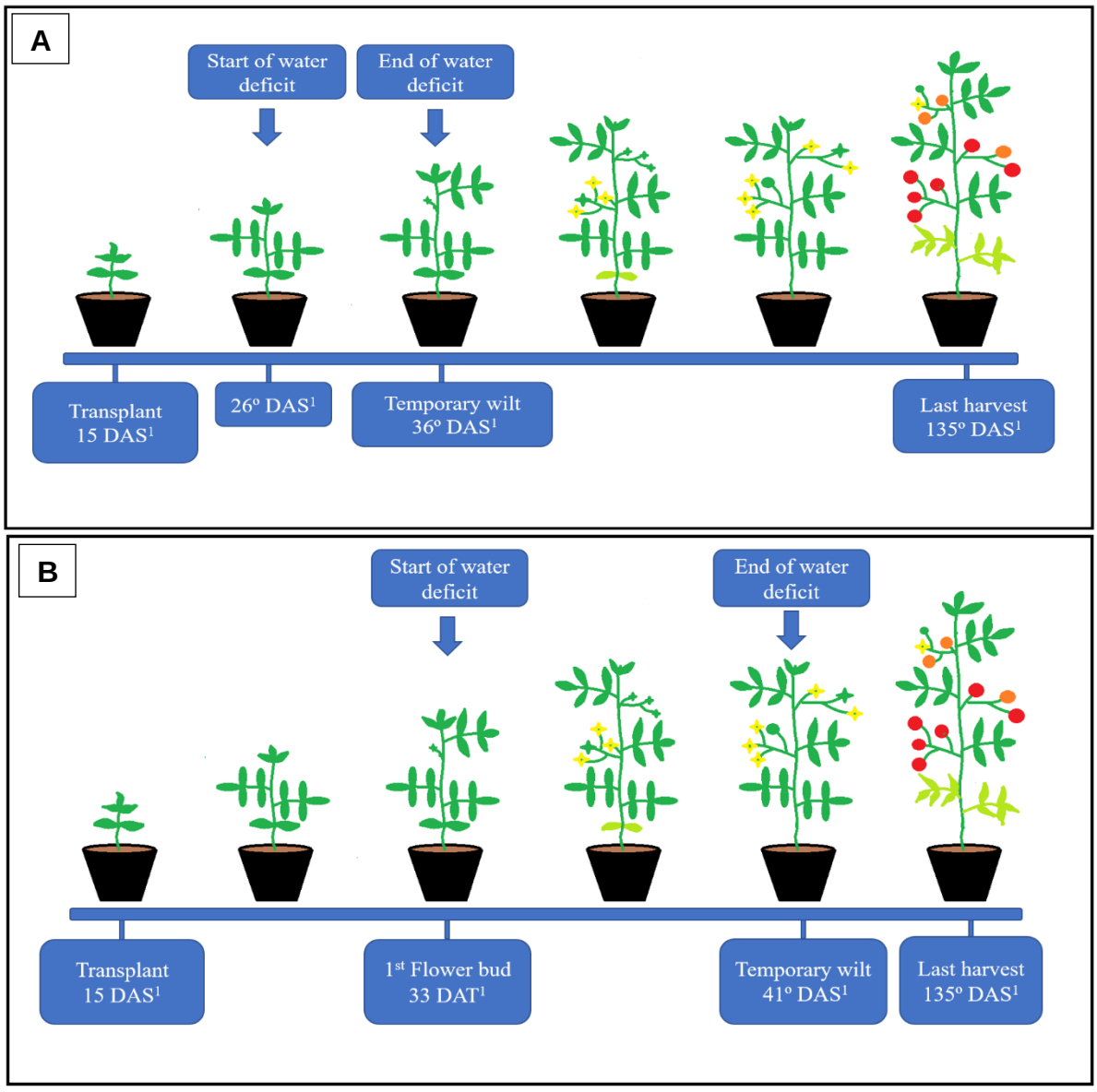

Figure 1. Summary of the experimental design in which: (A) water deficit treatment was performed in vegetative stage growth, beginning at 26 DAS[1] and ending at 36 DAS[1] (A); (B) water deficit treatment was performed in reproductive stage growth, beginning at 33 DAS[1] and ending at 41 DAS[1]. Before and after the deficit period the pots were irrigated daily in order to maintain the soil in field capacity until the last harvest at 135 DAS[1]. [1]DAS: days after sowing.

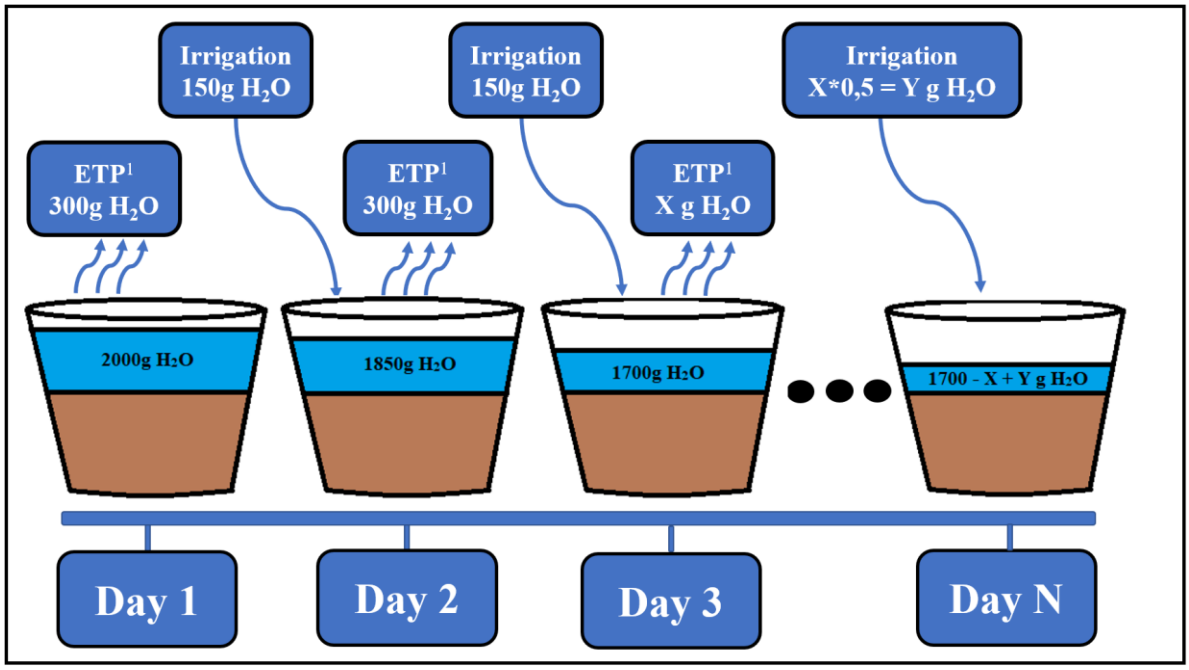

Figure 2. Model to replacement of 50% ETP during the 10 days in the vegetative growth stage and 8 days in the reproductive growth stage. The treatment of 100% ETP was maintained well-watered during whole experimental period. [1]ETP: evapotranspiration.